A Study of the Factors which Affect the use of Biological Indicators as Monitors of Lead

A thesis presented in partial fulfilment of the requirements for the degree

of

Doctor of Philosophy in Chemistry

by

Nigel G. Purchase

University of Canterbury

1983

Abstract

A study of two biological indicators was undertaken to investigate the factors that influence the levels of heavy metals as indicated by these biological indicators. The two indicators investigated were the shellfish Chione (Austrovenus) stutchburyi, which was used as an indicator of lead and cadmium in the Avon-Heathcote Estuary, Christchurch, New Zealand, and human teeth, which were used as an accumulative indicator of lead exposure to humans.

The factors that were found to affect lead concentrations in the shellfish chosen were size and breeding cycle activity. A temporal study of lead concentrations in these shellfish established that the levels of lead were determined by rainfall over Christchurch and the source of lead was identified as being the waste products of a small lead smelter and battery factory on the banks of the Heathcote River. It was shown that lead accumulated in the shells of the shellfish by both incorporation (during shell growth) and isomorphous replacement after surface absorption.

The factors that were found to influence the lead levels in permanent teeth were tooth type, tooth age, type of tooth material analysed and area of tooth from which sample was taken. Lead concentrations measured in whole or partial sets showed that lead levels reflect tooth age. A survey comparing suburban and rural children demonstrated that rural children had lower teeth lead concentrations but that this difference was not statistically significant,

though for inner city children the difference was significant.

A study of lead in river sediments showed that lead tended to be localised at points of entry and its sources were storm water run off and industrial waste from a battery factory. The chemistry of lead in the sediment was found to be determined by sediment pH and the redox conditions of the sediment. Also, an investigation of sorption of lead onto river sediments was carried out.

Contents

Contents cont.

Contents cont.

Contents cont.

Contents cont.

Contents cont.

Contents cont.

Contents cont.

Contents cont.

List of Tables

List of Tables cont.

List of Tables cont.

List of Tables cont.

List of Tables cont.

List of Tables cont.

List of Figures

List of Figures cont.

List of Figures cont.

List of Figures cont.

List of Figures cont.

List of Figures cont.

Chapter 1

An outline of the Objectives of this Study

The use of heavy metals by man has always brought with it the risk of unintentional poisoning, and as their industrial and domestic uses grow then the risks associated with these metals become more acute. It therefore becomes the aim of the analytical chemist to measure the levels of these metals in the environment in a meaningful way which relates both the toxicity and availability of these metals to man.

Interest in heavy metal availability is centred in two areas. The first is the investigation of levels of heavy metals in the environment in ways that can enter the food chain, and hence reach human beings. The second is attempting to measure the past exposure of people to heavy metals, in due course to see whether the exposure is producing any harmful health effects. The heavy metals of greatest concern, at the present moment, are mercury, cadmium and lead and while mercury and cadmium have caused several environmental and health disasters because of industrial discharge, the third element, lead, is of greatest concern.

Lead is of great interest because it is the most widely used and distributed of the three heavy metals and its present so called "normal" levels in human beings are close to those where signs of clinical poisoning would be diagnosed. For this reason lead is the main element studied in the present work. Lead is widely spread in the environment and with its former use in paint, water piping, solder and glazes and its continued use as an anti-knock agent most

people are exposed to it. While the effects of clinical lead poisoning are well known and accepted, the subclinical effects of lead are still the subject of much controversy. It is in this latter area that much intensive research is being carried out.

While it is not the primary task of the analytical chemist to determine if there are effects on the health of people it is, however, the task of the analytical chemist to supply accurate and reliable data on the levels of trace elements present within the environment, or within people, and provide them in a clear and unbiased fashion. However, the analytical chemist is presented with the problem of how to meaningfully assess the levels of pollutants present.

One method chosen to do this is with the use of biological indicators and the aim of this study is to look at two biological indicators that have been widely used. The first of these is the use of shellfish to monitor pollution in the marine environment and the second is the use of human teeth as a monitor of exposure of humans directly to pollutants. These two biological indicators are representative of accumulative indicators, which are used to measure total exposure of an organism to a pollutant, and temporal indicators which provide information on the average exposure of an organism to a pollutant over time.

Shellfish were investigated as temporal indicators. While levels of trace elements measured in shellfish have been widely used to indicate levels of pollution within the vicinity of the shellfish (1-4), it has been found that several environmental and physiological factors influence the organism's ability to provide accurate pollution levels

(5-7). The aim of this study was to use the soft parts of the shellfish as indicators of lead pollution within the Avon-Heathcote Estuary (a small intertidal estuary near the city of Christchurch, New Zealand). The analytical problems are twofold. Firstly, a method of analysis had to be found which was capable of producing accurate levels of the quantity of lead present, and secondly, a study had to be designed in order to check on which physiological and environmental factors were affecting the measured results. A further aim of this study was to investigate factors which affected the levels of lead within the estuary and if possible to identify the source of lead pollution to the estuary.

Two accumulative indicators were investigated in this study, the first being the shells of shellfish and the second being human teeth. The study of shells was directed towards seeing how pollutants become incorporated into the shell structure, and investigations of environmental factors that affect the shell's ability to act as an indicator.

The second accumulative biological indicator studied was human teeth. This study was primarily aimed at looking at the problems of sampling and the effect of different samples on indicator pollutant levels. This is of importance as teeth have been used in studies to determine if subclinical effects of lead poisoning can be related to human performance (8-10). Factors such as tooth age, tooth type and the part of the tooth analysed were considered in relation to tooth lead concentrations. This work was done on permanent teeth and it appears that the results are in agreement with those obtained with deciduous teeth.

Having obtained an understanding of lead accumulation in teeth then a survey type study was carried out with the express aim of seeing whether rural children living in Canterbury, New Zealand, had a different level of lead exposure to those living in urban Christchurch, by looking at the levels of lead found in their deciduous teeth. In order to relate the present work to previous studies a review of lead levels in teeth is also presented.

As a result of the studies on the shellfish, it was considered advisable to investigate the chemistry and behaviour of lead within the Avon-Heathcote River system. Firstly, a survey of surface sediments was undertaken to find the areas where lead was being introduced into the sediments, and secondly, a study was made of how lead behaved in these sediments. The behaviour of lead within the sediments was investigated in two ways. The first of these was an investigation of the sorption of lead onto river sediments to give some insight into the method of fixing lead to the sediments, and the second study was an investigation of the chemistry of lead within the sediment and looking at what factors influenced the form of lead compound found.

References.

1. Phillips, D.J.H. The common mussel Mytilus edulis as an indicator of pollution by zinc, cadmium, lead and copper. II. Relationship of metals in the mussel to those discharged by industry. Mar. Biol., 38, 71-80, (1976).

2. Chow, T.J.; Snyder, H.G.; Snyder, C.B. Mussels (Mytilus sp.) as an indicator of lead pollution. Sci. Total Environ., 6, 55-63, (1976).

3. Nielsen, S.A.; Nathan, A. Heavy metal levels in New Zealand molluscs. N.Z. J. Mar. Freshwater Res., 9, 467-481, (1975).

4. Golberg, E.D.; Bowen, V.T.; Farrington, J.W.; Harvey, G; Martin, J. H.; Parker, P.L.; Risebrough, R.W.; Robertson, W; Schneider, E; Gamble, E. The mussel watch. Environ. Conserv., 5, 101-125, (1978).

5. Phillips, D.J.H. The common mussel Mytilus edulis as an indicator of pollution by zinc, cadmium, lead and copper. I. Effects of environmental variables on uptake of metals. Mar. Biol., 38, 59-69, (1976).

6. Latouche, Y.D; Mix, M.C. The effects of depuration, size and sex on trace metal levels in bay mussels. Mar. Pollut. Bull., 13, 27-29, (1982).

7. Simpson, R.D. Uptake and loss of zinc and lead by mussels (Mytilus edulis) and relationships with body weight and reproductive cycle. Mar. Pollut. Bull., 10, 74-78, (1979).

8. Needleman, H.L.; Gunnoe, C; Leviton, A; Reed, R; Peresie, H; Maher, C; Barrett, P. Deficits in psychologic and classroom performance of children

with elevated dentine lead levels. N. Engl. J. Med., 300, 689-695, (1979).

9. Winneke, G; Hrdina, K-G; Brochkaus, A. Neuropsychological studies in children with elevated tooth lead concentrations. Part I: Pilot study. Int. Arch. Occup. Environ. Heath, 51, 169-183, (1982).

10. Winneke, G; Kramer, U; Brockhaus, A; Ewers, U; Kujanek, G; Lechner, H; Janke, W. Neuropsychological studies in children with elevated tooth lead concentrations. Part II. Extended study. Int. Arch. Occup. Environ. Heath, 51, 231-252, (1983).

Chapter 2

Marine Pollution Monitoring by Biological Organisms

2.1.1 Introduction

The monitoring of marine and estuarine pollution for heavy metals has received considerable attention recently. This concern is not just purely environmental, as man has been consuming fish and shellfish from the seas for years. However, increased industrialisation around coastal areas has occurred, threatening the purity and cleanliness of our coastal marine resources. There is therefore a need to determine the level of pollution in affected areas.

The traditional methods for measuring heavy metal pollution in marine systems have been water and sediment monitoring. The analysis of water samples, however, suffers from both analytical and sampling problems. The analytical problems occur because of the low level of pollutants in water, for example, levels of lead in seawater may be as low as .02 μg Pb/L (1). This places strain on the ability to remove contaminants (such as in reagents) which could affect or swamp the true levels of pollutants. Few analytical techniques are accurate at these levels, therefore it is necessary to include some form of preconcentration which itself may affect the accuracy of the measurements. The problems of sampling water for analysis, especially in estuarine systems, occur because of the continuously changing water flow. This causes difficulty when comparing results for different areas and

for different times of sampling. Also the level of water input can critically affect the results, for example, flooding of rivers, or low rainfall can drastically change the results obtained at different times.

The second method of traditionally analysing for metal pollution in estuarine and marine systems is the analysis of sediments for their metal contents. Here the concentrations are higher, allowing for more conventional methods of analysis to be undertaken, reducing somewhat the problem of external environmental contamination. The disadvantage of sediment analysis, however, is interpreting the results in terms of the availability of pollutants to the system under investigation and ultimately to man, as many metal pollutants are strongly bound to the sediment and are unavailable to the biological components of the system. Thus this point of bio-availability makes sediment analysis somewhat suspect as to an indication of pollution potential.

2.1.2 The "Ideal Indicator"

Because of the problems outlined above, scientists are searching for the "ideal indicator". This indicator would be required to integrate the level of pollution in the system, hence smoothing the peaks and troughs of water analysis. The indicator would have to be able to survive the rigours of its environment. Also it would be required to respond only to the type of pollution to be investigated, and to respond in a linear or at least predictable way to change in the pollution load at an easily analysed level.

2.1.3 Marine Biological Indicators

The search for the "ideal indicator" has focused on biological indicators, as it has been shown that certain marine organisms accumulate heavy metal pollutants from aqueous solution. This provides material with trace metals at a suitable concentration level.

A variety of bio-indicators have been used in marine pollution monitoring. These fall into three categories: (i) Shellfish, including barnacles, gastropods and bivalves. (ii) Algae and plants. (iii) Finfish.

2.1.4 Prerequisities of a Good Bio-Indicator

Before commencing monitoring studies on aqueous heavy metal pollution it is necessary to carefully consider the characteristics of both the organism to be used to monitor the pollutant and the nature of the pollutant. The following list of prerequisites for a good bio-indicator has been proposed by previous authors (2).

(a) The organism should accumulate the pollutant without being killed by the levels encountered in the environment.

(b) The organism should be sedentary in order to be representative of the study area.

(c) The organism should be abundant throughout the study area.

(d) The organism should be sufficiently long-lived to allow the sampling of more than one year class if desired.

(e) The organism should be of reasonable size giving adequate tissue for analysis.

(f) The organism should be easy to sample and hardy enough to survive in the laboratory allowing defecation before analysis (if desired), and laboratory studies of pollutant uptake.

Later the following additions were made (3):

(g) The organism should tolerate brackish water.

(h) A simple correlation should exist between the pollutant content of the organism and the average pollutant concentration in the surrounding water.

The final requirement was later amended (4, 5):

(h) All organisms of a given species used in a survey should exhibit the same correlation between their pollutant content and the average pollutant concentration in the surrounding waters, at all locations studied and under all conditions.

Later, consideration will be given to how well the indicator organism chosen for this study meets these criteria. But first, a brief summary will be given of factors which have influenced previous work with bio-indicators.

2.1.5 Factors Which May Affect the Interpretation of Results from Bio-Indicators.

The following factors have been found to affect the relationship between the pollution in the environmental system and the levels of pollutants as measured in the bio-indicators. These factors will also be considered later when the results of this work are discussed.

2.1.5 (a) Seasonal Variation in Trace Metal Levels in Marine Bio-Indicators.

Seasonal variation in the observed metal concentrations in bio-indicators raise problems for the two most common uses of bio-indicators, which are the intersite comparisons and changes in pollutant levels at one site over a period of time. Seasonal variation can be divided into three categories:

(i) Seasonal variation in the delivery of pollutants.
(ii) Organism physiology, particularly the sexual cycle.
(iii) Changes in ambient water quality parameters such as salinity, temperature and organisms' food supply.

Since heavy metals come predominantly from industrial sources it is normal to expect a constant input flux of pollutant. However, in some cases seasonal rainfall can influence the results either by increasing the delivery rate of pollutants, or by diluting the pollutants.

An organism's physiology can undergo seasonal variation

which may affect the measured pollutant load. As the pollution load is generally calculated as the concentration of heavy metal per weight of animal, any rapid change in animal weight will affect the observed concentrations. In areas of temperate climate many marine organisms have both a pronounced breeding season and a pronounced growth season. During the breeding season many animals increase their body weight by as much as 40%, over a period as short as two months. Also it appears that this gonad material contains less heavy metal than the rest of the animal. Hence, this causes an apparent lowering of levels during gonad production with an apparent increase in levels after the release of gonad material.

A similar effect, but generally on a much smaller scale, can occur if the organism undergoes a rapid growth phase over a short period of time, that is, if the organism's growth rate is greater than its rate of accumulation of pollutant.

In some marine systems there are seasonal changes such as salinity, water temperature and available food supply for organisms living in the system which may also affect the results of any heavy metal/animal concentration studies. Generally these changes affect the stress the animal lives under. For instance, in estuarine systems if the water run off decreases, the salinity will tend to increase. For some animals this is a less favourable environment and hence the growth rate drops. This may also lead to an apparent metal concentration increase in the animals. The effect of temperature on growth is such that in certain areas no growth occurs at all in some species if the water temperature drops below a certain value.

Seasonal variation in food supply can, however, produce the greatest fluctuation in measured pollutant/animal concentration. In times of high food availability, and providing the food is not the chief source of pollutant, growth may be rapid, (generally true only for young and immature organisms), reducing the apparent metal/animal concentrations. However, if food becomes scarce the animal is forced to use up its own body store lowering its weight and, depending on the rates of accumulation/excretion of pollutant, may show an increase in pollutant/animal concentration.

The most common way to overcome these problems, if intersite comparisons are being made, is to sample all sites over a narrow time range. The time of year for this sampling should be chosen to minimise the influence of seasonal factors, that is, at a time when the system is in some resemblance of equilibrium.

2.1.5 (b) The Effects of Age (Size, Weight) on Trace Metals in Marine Bio-Indicators.

As any organism grows, its physiology can change and this can influence its ability to act as a bio-indicator. If, for example, at some stage before adulthood an organism depended predominantly on one food source and then later switched to another food source, this could affect the amount of pollutant the organism was digesting.

For the "ideal indicator" there would be no variation in heavy metal concentration with change in animal weight due to age. However, from previous studies (4, 6, 7) it

has been shown that for some chemical elements and some species there is a marked size (or age) concentration relationship. It was once suggested (8) that the size/concentration relationship was invariant with regards to site choice. However, it now appears that each population of a species may have different size/concentration relationships at different sites or at different times of the year.

Different reasons have been given for this phenomenon, and include changing food demands with age, growth rates affecting pollution accumulation, influence of sexual behaviour and the past history of the organism. The growth factor occurs because an immature organism is still growing and may be more susceptible to incorporate pollutants within itself or may be less able to remove them. The mature organism, on the other hand, has probably reached its full size or only grows slowly and spends most of its energy in producing gonad which appears to be selective against the incorporation of pollutant material.

To cover the problem of concentration/size (age) variation amongst differing populations of a species, four alternatives are used when comparing populations. Either,

(i) the effects of size are ignored, or
(ii) organisms of the same, or similar, sizes are taken from each population studied, or
(iii) organisms having a large range of sizes are selected and analysed in bulk, or
(iv) organisms of a large range of sizes are selected and analysed individually to generate a size/concentration regression for each population.

Option (i) can produce in some cases, depending on the severity of the size/concentration effect, erroneous results because of different sizes being dominant in different populations. Option (ii) raises problems in that the size range may have to be strongly restricted in some cases, and this can cause sampling problems as some populations may not contain specimens of the required size. Option (iii) raises difficulties if the mean population weights of the two or more samples which are being compared, are not closely matched. Option (iv) has the problem that a large number of specimens must be analysed from each site to build up a true picture of the size/concentration effect and this may involve a large expenditure of time and resources.

The best compromise is probably Option (ii) but it is necessary to realise that it does not give a complete picture of the overall population and the limitations involved in obtaining samples.

2.1.5 (c) Pollutant Interactions as a Source of Error in Monitoring Studies.

This source of error arises when the concentration of an element under investigation is influenced by another component in the system. This can be external or internal. For example, the element can form with other materials in the environment, a stable species so that the organism is no longer exposed to the element. This will lower the apparent concentration of pollutant in an area as measured

by the analysis of the bio-indicator organism. Similarly if elements compete for the same sites within the organism then one element may be preferentially excreted, hence the levels of pollution as measured in the bio-indicator will be low.

2.1.6 Choice of Indicator Organism for this Investigation.

This study looks at the flow of pollutants and their apparent concentrations in a small estuarine system. The nature of the system being investigated, and the requirement to look at specific sites within the system, ruled out the use of mobile species such as finfish and crabs. Because the growth of weed within the estuary varied greatly this was also ruled out.

Hence the requirement for stable, non-mobile populations led to the use of shellfish. In the system that was studied the shellfish Chione (Austrovenus) stutchburyi, or cockle, was widely distributed and used in this work.

2.1.6 (a) Description of Chione (Austrovenus) stutchburyi (Wood 1828).

Chione is a filter-feeding bivalve mollusc. It belongs to the family veneridae. Its common name is the cockle and it has the Maori names Huangi and Tuangi. It lives in the North, South, Stewart and Chatham Islands of New Zealand. This bivalve meets many of the requirements for a bio-indicator as laid out in section 2.1.4. It was reported

to accumulate heavy metals (9-13) and being a filter feeder it is relatively immobile (14). In the study area it is abundant, approaching in some areas a density of $3000m^{-2}$ (14). Shellfish were found with ages up to 10 years and having a dry weight of between 0.3 - 1.0µg which was sufficient for easy handling. The cockle, being a sediment dwelling filter feeder, is relatively simple to obtain, living in the top 5 cm of sediment and it is hardy enough to survive for more than 48 hours in marine water in the laboratory. The requirement to be able to survive in brackish water was not necessary and the final requirement of having a fixed correlation between the marine pollutant load and the minimal body load was to be tested in this work.

Although Chione appears to meet most requirements for a bio-indicator, because it does not inhabit areas outside of New Zealand there is little previous data available to compare with this work.

2.1.7 Description of the System Investigated.

The system investigated was the Avon-Heathcote Estuary near Christchurch New Zealand.

The Avon-Heathcote Estuary is a small ($6km^2$ area), shallow (mean depth at high water on spring tide = 1.4 m), bar built estuary. It is largely intertidal, (85% of the estuary is intertidal mudflat), with almost complete tidal exchange. It has a drainage basin of approximately 200 km^2, over half of which is the urban area of the city of

Christchurch (population approximately 300 000) see Figure 2.1.

2.1.8 Intentions of this Study.

The intentions of this study can be divided into two areas, namely an investigation of the suitability of Chione as a bio-indicator and the use of Chione as a water pollution bio-indicator.

2.1.8 (a) Suitability of Chione (Austrovenus) stutchburyi as a Bio-Indicator.

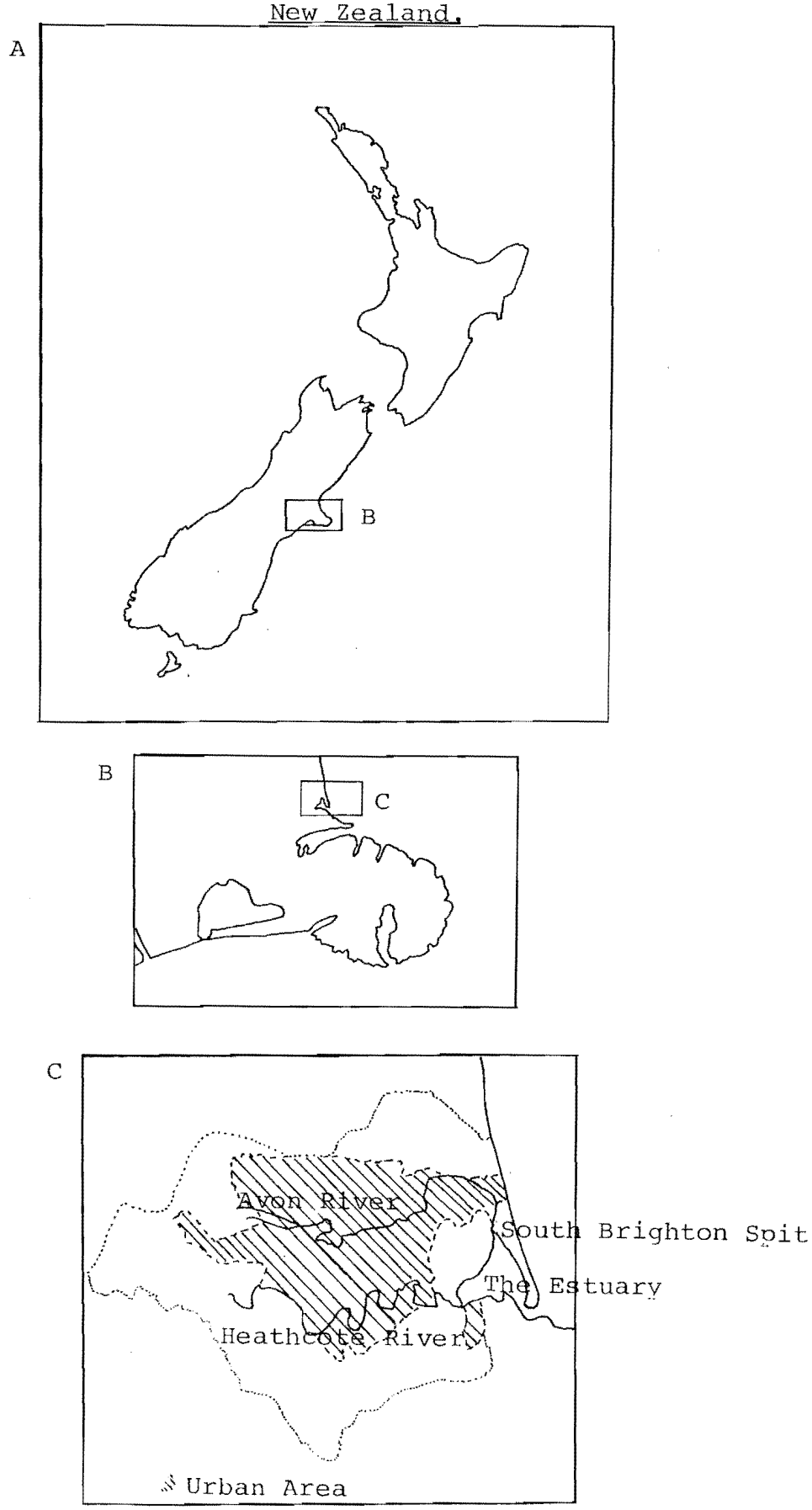
In this part of the study an investigation was made into some of the factors that influence the concentration of pollutants in the shellfish. This included an investigation into the effect of size (age and weight), the breeding cycle and the distribution of pollutant within the animal, to see if accumulation occurred in any particular organs or was uniformly distributed throughout the animal.

2.1.8 (b) Use of Chione (Austrovenus) stutchburyi as a Water Pollution Bio-Indicator.

This part of the study was divided into two sections. Firstly a study of the changes in pollutant levels over time. The site chosen was considered to be sufficiently removed from localized pollutant sources, that the levels

Figure 2.1

Location of the Avon-Heathcote Estuary, Christchurch, New Zealand.



were considered representative of the total system. This site was sampled monthly over more than a three year period. The second part of this study was a partial survey of the geographical variation of pollution within the area. This involved the sampling of different areas at the same time (so as to minimise seasonal effects) in order to look for changes due to site.

2.1.9 Pollutants to be Studied.

Before the study was undertaken a decision was made concerning constraints of time, equipment, and which elements should be investigated. At the time, considerable interest was aroused by a report (10) that suggested that lead from automobile emissions was being accumulated by Chione. It should be noted that New Zealand has one of the highest levels of lead anti-knock agent (0.84 g Pb/L^{-1}) added to its fuel in the world. One other source of lead to the system existed viz. a lead battery factory on the banks of the Heathcote River, 45 km from the Estuary.

Therefore it was decided to study the levels of lead within the Avon-Heathcote Estuary to see if the metal was accumulating and whether in specific areas, and to see, if possible, whether the contributions from the two lead sources could be determined.

2.2 Analytical Methods

2.2.1 Collection and Handling Prior to Analysis.

Shellfish (Chione (Austrovenus) stutchburyi) were collected by hand from designated sites at low water. Approximately 15 specimens were obtained from a small area (generally about ½ m^2) and these placed in a previously washed glass jar. The shellfish were washed in clear estuarine water obtained near their sampling site to remove any sand or sediment from their external surfaces. The jar was then filled with clear estuarine water, sealed and taken to the laboratory.

The specimens were kept undisturbed for 1-2 days to allow for defecation of any sediment or waste. The shellfish were removed from their shells with the use of stainless steel single edged razor blade or surgical scalpel and placed into acid washed pyrex glass beakers, total animal weight, shell weight and wet weight of the animal all being recorded.

The shellfish were then maintained at 80°C for 24 hours in a clean drying box, after which the dry weight was recorded. By this time a steady weight was obtained, there being no further significant weight loss after a further 24 hours at 80°C.

2.2.2 Processes for Ashing of Shellfish.

Two methods were used for the ashing of shellfish prior to analysis, that is wet ashing and dry ashing.

2.2.2 (a) Wet Ashing.

The shellfish were treated with 10 mL of a 3:1 mixture of concentrated nitric and perchloric acids and heated slowly until fuming had ceased. The residue was then taken up in 10 mL of 2M nitric acid, heated until all material had dissolved and when cool transferred to a volumetric flask (25 mL).

This method was later abandoned after attempts at checking the accuracy by the method of standard additions. It was found that not all of the organic matter had been destroyed and the residual organic material gave rise to non-atomic absorption when the analysis was carried out by flameless atomic absorption spectrophotometry. This was confirmed when with the use of a deuterium continuum lamp the signal was found to be almost entirely due to background scattering or absorption.

2.2.2 (b) Dry Ashing.

Shellfish samples, still in their borosilicate beakers, were placed in an ashing oven at 400^{o}C for 12 hours. The residue was taken up in 5 mL of 2M HNO_3 (boiled for 30 minutes) then filtered through Whatman 40 filter papers, which had been prewashed with 0.5M HNO_3.

The recovery using this method was determined to be between 97 - 102%. An earlier attempt using silica crucibles was discarded when the recovery was found to be as low as 20 - 30%. It was believed that there may be some fixation of lead ions to the silica surface that could not be recovered by acid washing.

2.2.3 Instrumentation and Analysis.

The analysis of lead and cadmium in shellfish (Chione) was carried out by graphite furnace atomisation atomic absorption spectrophotometry. (The terms graphite furnace atomisation and flameless are used interchangably in this study). The atomic absorption spectrophotometer initially used was a Varian Model AA-5 fitted with an optional background corrector, this being later replaced by a Varian Model AA-1475. No difference was noted in the results using either machine. The graphite rod furnace was a Varian Model CRA-63, which gave the choice of either carbon rod or carbon cup atomisation. In this study graphite rod atomisation was used throughout because of its greater sensitivity (≃2 fold).

2.2.4 Instrument Settings.

The following instrument parameters were used throughout the study.

Varian Model CRA-63 Graphite Furnace Atomiser.

Mode	Voltage Setting	Time Setting	(approx. time)
Dry	4.0	15	30 sec.
Ash	5.0	5	10 sec.
Atomise	7.0	Ramp 400°C/sec	

In the atomise phase the ramp mode was used because even though the ashing removed all organic matter some inorganic ash remained, and since background correction was found not to be very satisfactory, ramping allowed for the isolation of the lead peak.

The instrument parameters used on the atomic absorption spectrophotometers are given in Table 2.1.

Although the Varian AA-1475 has a double beam facility, this was not used due to the halving of the available signal, and hence raising of the noise threshold.

The absorbance was outputted directly onto graph paper and the absorbance obtained by measuring the peak height.

2.2.5 Accuracy of Method.

The accuracy of the analytical method was tested in two ways. Firstly, the method of standard additions was used on some samples. The results were the same as those obtained by direct comparison with a standard curve obtained by the addition of measured amounts of lead to 0.5M HNO_3 solution.

A further check on the accuracy was obtained by comparing the results for the same samples using the above method and anodic stripping voltammetry. With ASV it was found necessary to correct for interference effects by the method of standard additions. A list of the two sets of results is given in Table 2.2. No significant difference exists between the two sets of results.

2.2.6 Reagents and Preparation of Glassware.

Because of the low levels of the pollutants being measured in this study, contamination had to be kept to a minimum. To achieve this all glassware was soaked in a non-ionic detergent in distilled water for 12 hours,

Table 2.1

Instrument Settings.

	AA-5	AA-1475	
	Pb	Pb	Cd
Wavelength	217.0 nm	217.0 nm	228.8 nm
Lamp Current	5 mA	5 mA	3 mA
Slit Width	300 µm	1 nm	0.5 nm
Mode	Absorbance	Absorbance	Absorbance

Table 2.2

Comparison of AAS and ASV

Shellfish	CRA-AAS	ASV
1	0.76±.04	0.94±.02
2	0.85±.06	0.80±.04
3	0.40±.06	0.39±.04
4	0.35±.02	0.52±.02
5	0.14±.02	0.21±.02
6	0.72±.02	0.66±.02
7	1.19±.20	1.10±.02
8	0.54±.04	0.42±.03
9	0.37±.02	0.34±.03
10	0.35±.04	0.38±.02
mean±standard deviation	0.57±.31	0.58±.29

Notes: (1) Values are mean±error. (2) Lead Concentration in $\mu gPbg^{-1}$ dry weight.

then rinsed in distilled water, boiled for 30 minutes in 2M HNO_3 then rinsed again in distilled water and allowed to dry at $80^{o}C$.

The nitric acid used in this work initially was AJAX "AR" nitric acid. This was found to have a very low residue level of either lead or cadmium. However, later BDH "AnalaR" nitric acid had to be used and this was found to have a higher level of lead. In this case the acid was redistilled in an all glass still, giving a lead concentration of less than 5ng $PbmL^{-1}$, which was considered acceptable.

The standards were prepared from "AR" lead nitrate, which was heated prior to weighing and dissolved in 0.5M HNO_3 to give a 1000μgmL^{-1} stock solution. Working standards in the range of 25- 200 $ngmL^{-1}$ were prepared from this stock solution and 0.5M HNO_3. For cadmium, "AR" cadmium sulphate ($3CdSO_4.8H_2O$) was used to prepare a 1000μgmL^{-1} stock solution in 0.5M nitric acid and from this stock solution working standards in the range 5 - 50 $ngmL^{-1}$ (in 0.5M nitric acid) were prepared.

2.3.1 Results and Discussion

Because of the lack of extensive previous work carried out on the shellfish used in this study, the results are compared with those obtained on mussels and oysters. Both of these shellfish are filter-feeding bivalves.

2.3.2 Effects of Size (Age, Weight) upon Concentration.

In order to determine the ability of Chione to act

as a bio-indicator, it was first necessary to establish whether certain factors influenced the levels of pollutant concentration observed. The first factor investigated was the size (age or weight) of the sample animals. No reports have been published on Chione regarding the size/concentration effect. However, for the shellfish Mytilus edulis (4, 8, 15, 16, 17, 18), the oyster Ostrea edulis (7) and in some other shellfish (6, 19, 20, 21) there is a notable size/concentration relationship. There are, however, other interpretations of the results (21, 22).

Knowledge of the size/concentration relationship for Chione is important because the shellfish live in uniform age blocks, that is, at a particular site all the shellfish are of similar age and size, whereas at another site they may be a different size and age (14). Also, at sites which are not as favourable for Chione growth, the animals never approach the mature size.

The weight (age or size)/concentration relation is shown in Figures 2.2 - 2.4. All shellfish in this study were obtained from a site 250 m northwest of Beachville Road (see Figure 2.5). Three points may be noted. Firstly, while the regression coefficients (r^2) cover only 21-55% of the total variation between points there is a clear weight concentration relation. The large animals have, on average, a lower lead concentration than smaller animals. However, while smaller animals have higher concentrations of lead the overall total body burden of lead is greater in the larger animals.

Secondly, little variation in concentration occurs over the weight range of 0.5-0.9 g. This possibly reflects

Figure 2.2

Graph Showing the Variation of Lead Concentration with Shellfish Weight over the Period August-September 1980.

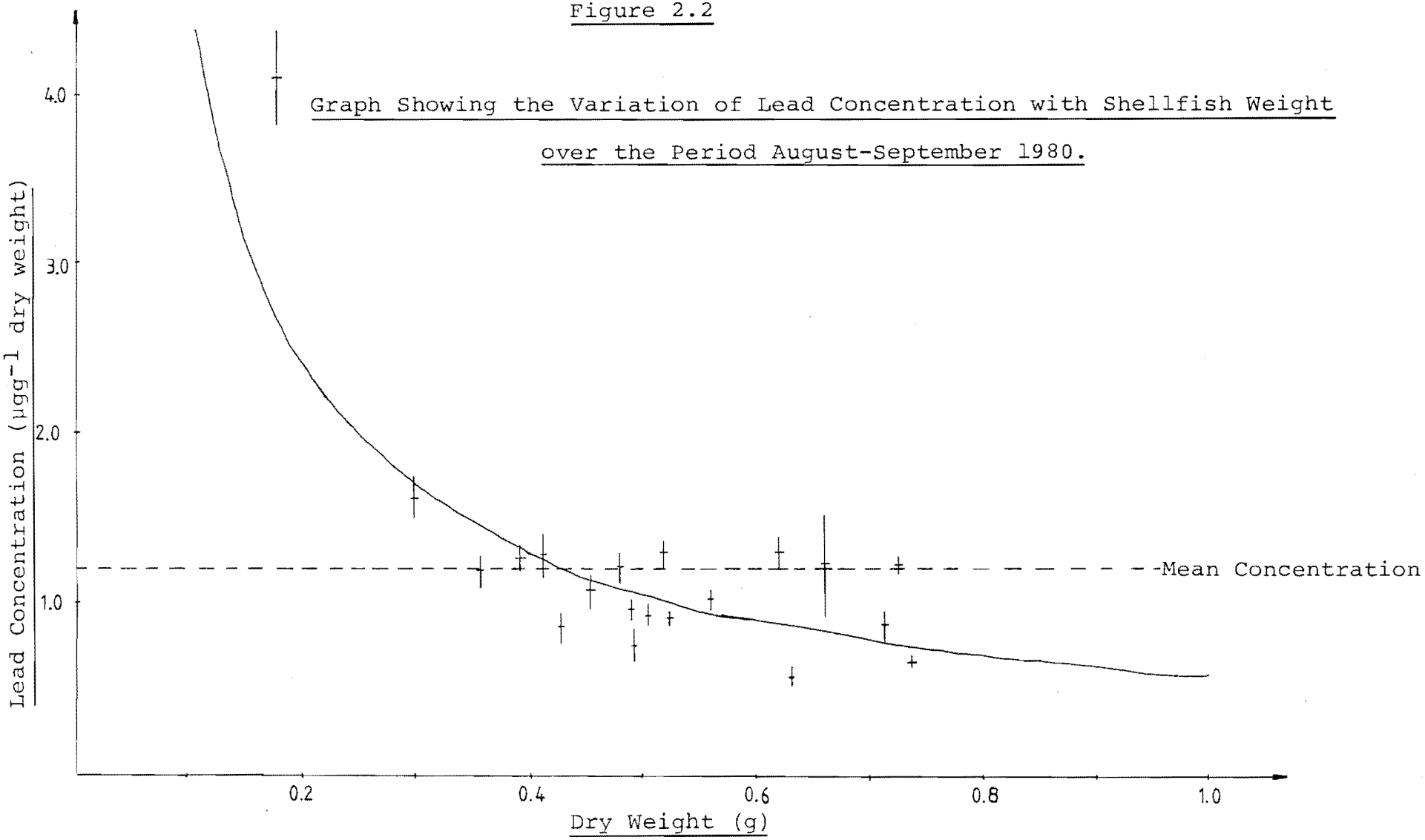


Figure 2.3

Graph Showing the Variation of Lead Concentration with Shellfish Weight Over the Period October-November 1979

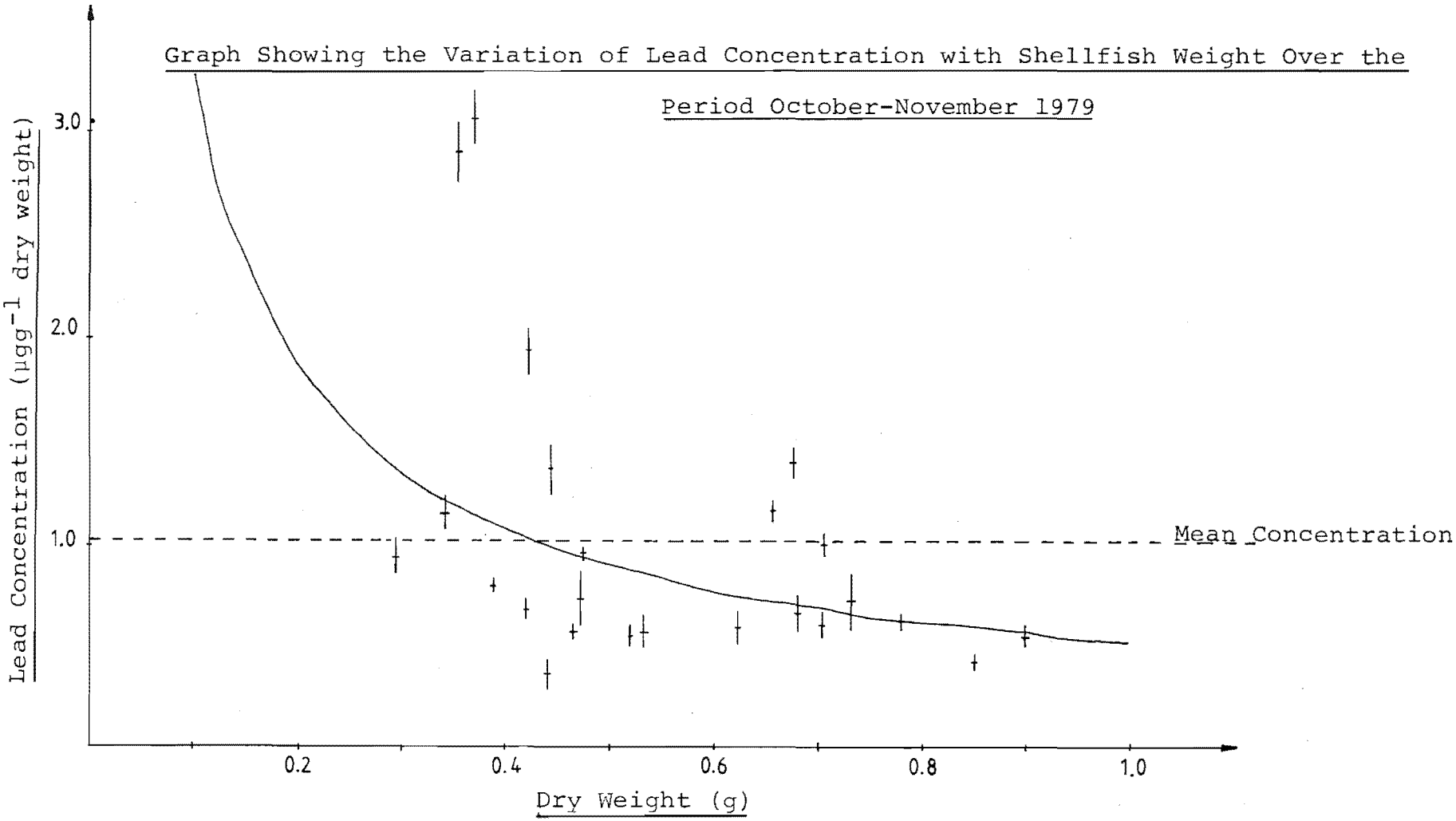


Figure 2.4

Graph Showing Variation in Lead Concentration with Shellfish Weight over the Period January-April 1981.

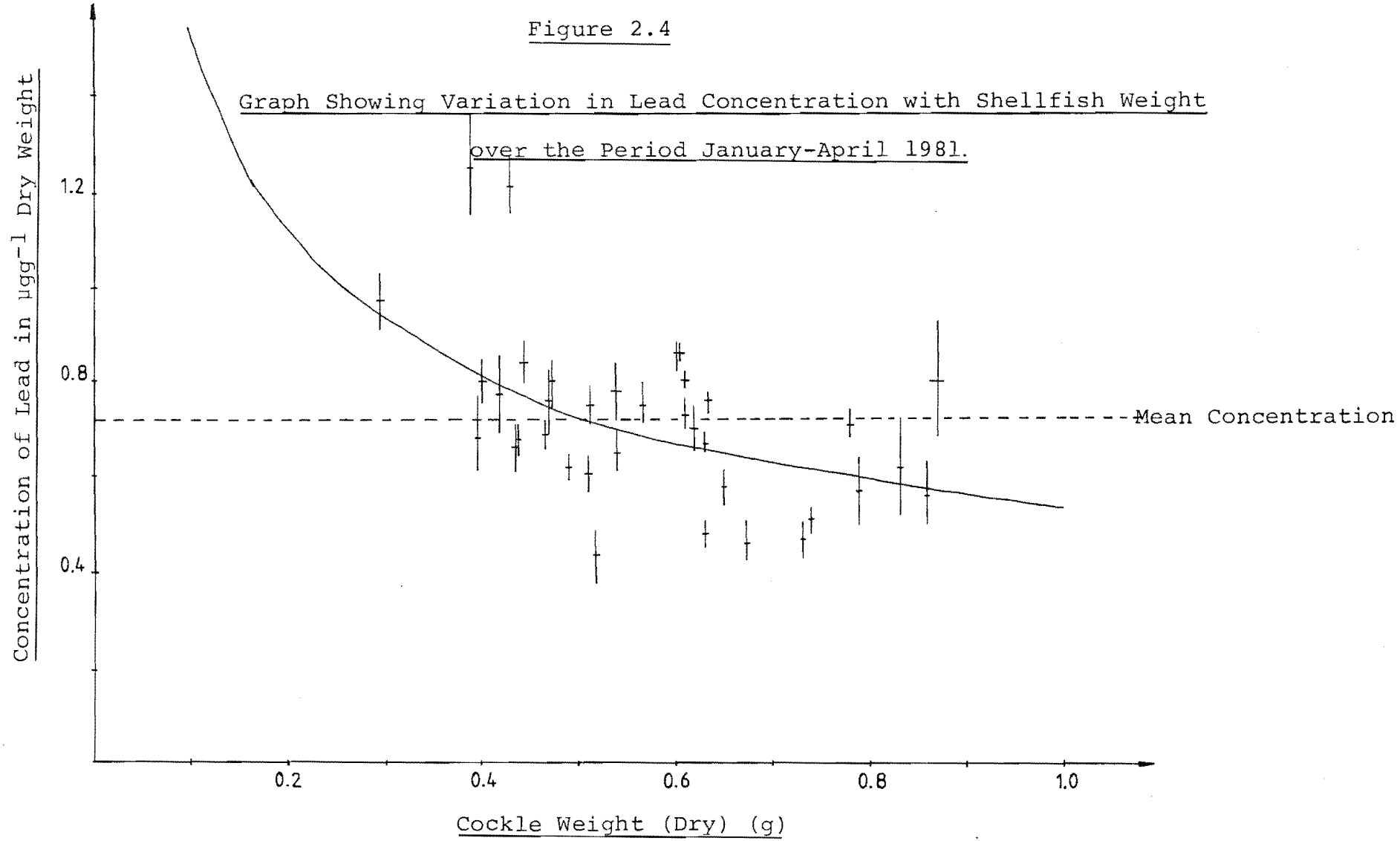
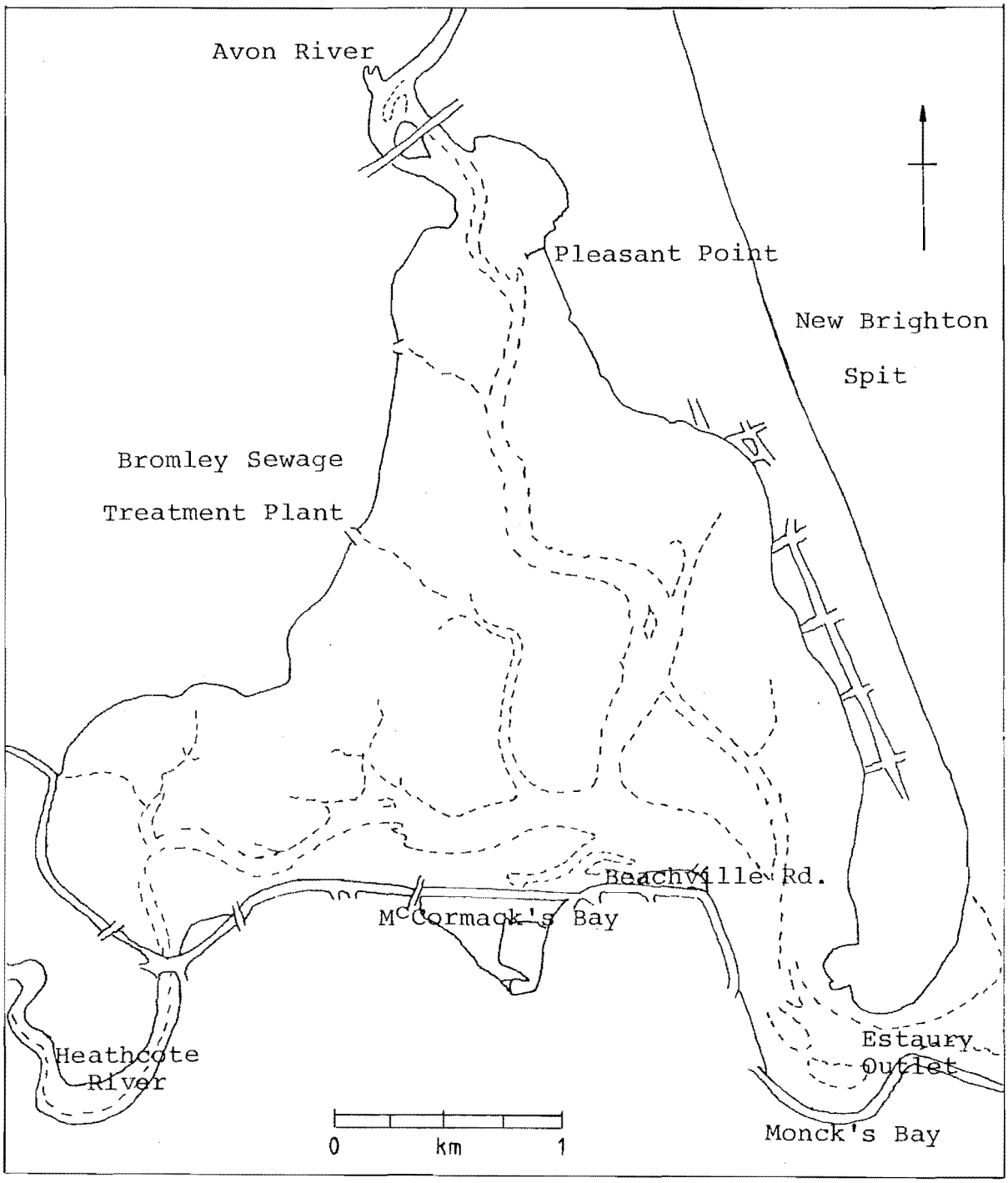


Figure 2.5

Map of the Avon-Heathcote Estuary.



the stable physiology of adult organisms compared with the immature organisms in the lower weight classes. The lower weight classes show greater variation over small weight changes, making this part of the population less than satisfactory for sampling work, as small weight differences could mask any difference due to environmental levels of lead.

Thirdly, even though the samples contributing to the data in Figures 2.2 - 2.4 came from the same site, the shape of the size/concentration relationship varies with the time of sampling. Part of the reason for the large variation in Figures 2.3, 2.4 is that the data in Figure 2.3 was obtained at the period when gonad was being formed and the difference between mature and immature shellfish is more marked at this time. The data in Figure 2.4 was collected for the January-April period when mature shellfish shed their gonad during spawning and this again causes variation between particular animal specimens. These results are in disagreement with the work reported by Boyden (8) on variation of concentration with size, as he believed the relationship did not vary. However, several other authors (4, 6, 17, 19) have noted a changing size/concentration relationship over time or site.

Hence the size/concentration relationship found raises the problem of interpretation of results from different sites. In this study one of the initial aims was to compare the levels of lead in McCormack's Bay with that of the rest of the estuary, as Millhouse (9, 10) had concluded that levels in McCormack's Bay were considerably higher than in the rest of the estuary.

To check this result shellfish were taken during the winter from both McCormack's Bay and the site approximately 250 m northwest of Beachville Road (see Figure 2.5). The results given in Table 2.3 appear to support the belief that the levels in McCormack's Bay shellfish are higher than in the estuary proper. In fact, by using the Student t-test it can be demonstrated that the difference in levels is significant at the $p<0.005$ level. However, when the data for both sites was plotted on a weight versus concentration graph, the best curve (on a log-log basis by linear regression) that fitted, passed through the data for the estuary site and the McCormack's Bay site (Figure 2.6). This raises questions about whether McCormack's Bay shellfish have a higher lead burden than for shellfish from the rest of the estuary.

The reason for the difference in animal sizes is that McCormack's Bay is separated from the main estuary by a causeway, which only allows exchange at high tide. As a result the salinity of McCormack's Bay is higher, and as the water flow is not as great in McCormack's Bay there is a higher percentage of mud in the sediment. As a result the sediments median grain size is smaller than the optimal value of 150μm for Chione (14) and the shellfish never reach as large a size as in the main estuary.

2.3.3 Effects of Sexual Cycle on Pollutant Concentrations.

Before monitoring studies can be carried out using shellfish as bio-indicators within a system, something has to be known about any natural seasonal or cyclic variations

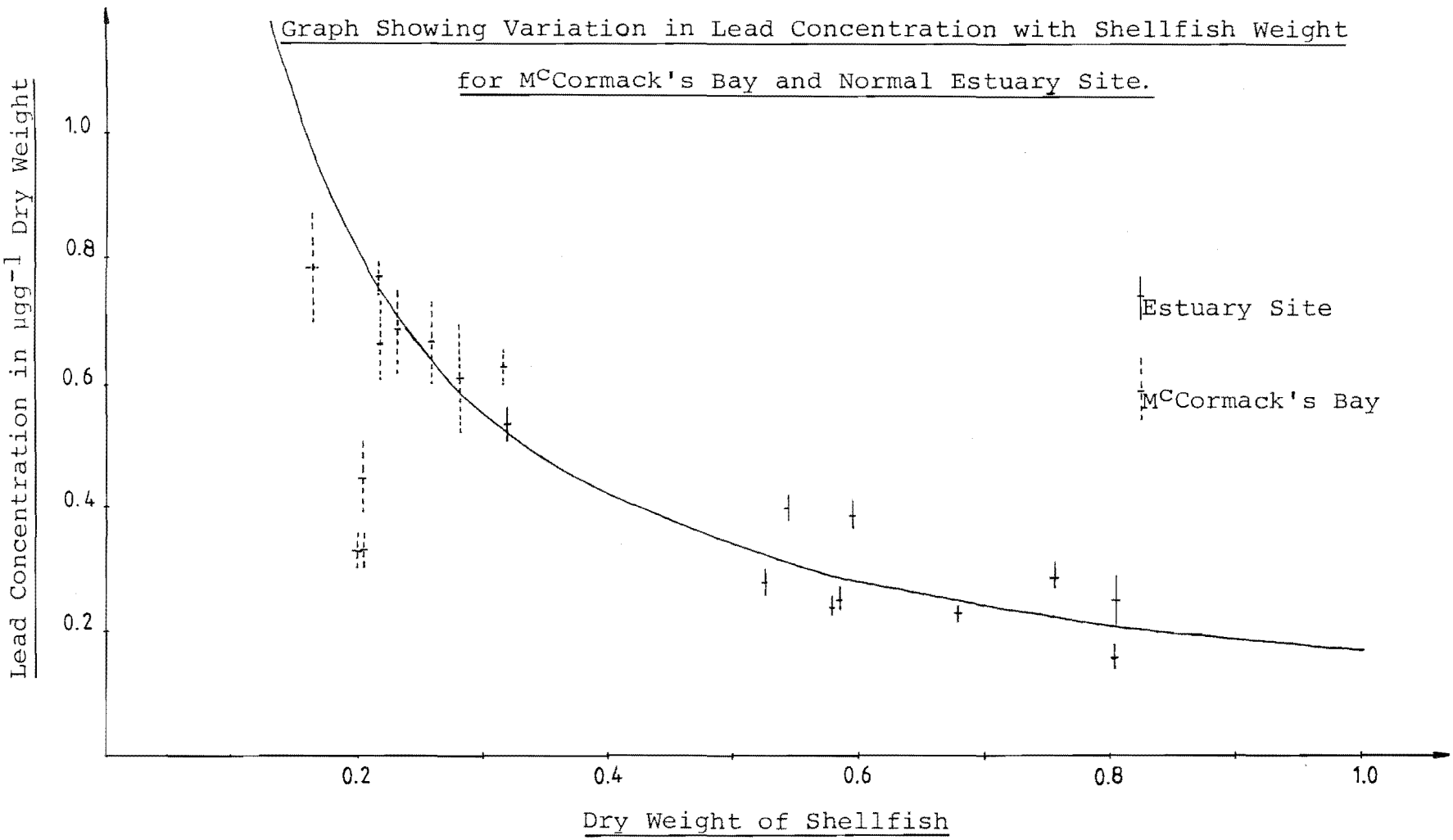
Table 2.3

Lead Concentration in Chione (Austrovenus) stutchburyi (in $\mu gPbg^{-1}$ dry weight).

	Estuary Site		M^{c}Cormacks Bay Site	
	Dry Weight	Concentration	Dry Weight	Concentration
1	.7573	.29±.02	.2838	.61±.09
2	.8048	.16±.02	.3210	.63±.03
3	.5254	.28±.02	.2442	.77±.03
4	.8064	.25±.04	.2182	.67±.07
5	.6790	.23±.01	.2608	.67±.03
6	.3184	.54±.03	.2334	.68±.07
7	.5435	.40±.02	.2077	.33±.03
8	.5986	.39±.02	.2049	.33±.03
9	.5844	.25±.04	.1698	.78±.09
10	.5552	.24±.02	.2055	.45±.06
Mean standard deviation		.30±.11		.59±.17

Note: (1) Values are mean±error.

Figure 2.6


Graph Showing Variation in Lead Concentration with Shellfish Weight
for McCormack's Bay and Normal Estuary Site.
Lead Concentration in μgg⁻¹ Dry Weight
1.0
0.8
0.6
0.4
0.2
0.2
0.4
0.6
0.8
1.0
Dry Weight of Shellfish
Estuary Site
McCormack's Bay

that occur for the shellfish. The most important of these is the sexual cycle. Variation in lead concentration which can be associated with changes in the reproductive cycle have been observed in mussels (4, 18, 24, 28) oysters (29, 30) and other bivalves (31). These are characterised by peak concentration values being recorded in the winter months and lower values being recorded in the summer months.

The graph in Figure 2.7 shows data for the concentration of lead in Chione (Austrovenus) stutchburyi for two successive breeding seasons. From approximately August-September the shellfish begin to accumulate gonad until December when it ripens, and generally spawning starts in January and finishes about the end of March (32). The data confirms that during the accumulation of gonad, lead concentrations decrease and then increase during the spawning part of the cycle. The suggested reason for this is that lead accumulation, and therefore the lead concentrations in gonad, are lower than for the rest of the soft parts of the body. The percentage of gonad present just prior to spawning can be as high as 40% (32).

It has been suggested that this phenomenon is just due to rapid weight gain with the total lead burden remaining constant, (4) causing an apparent concentration drop. But for the Chione at least, the data does not support this view as the concentration here appears to be weight invariant, although it should be noted that size was maintained throughout the collecting period to avoid problems as outlined in Section 2.3.2 (see Table 2.4).

Figure 2.7

Graph Showing Effect of Breeding Season on Lead Concentration in the Shellfish Chione (Austrovenus) stutchburyi.

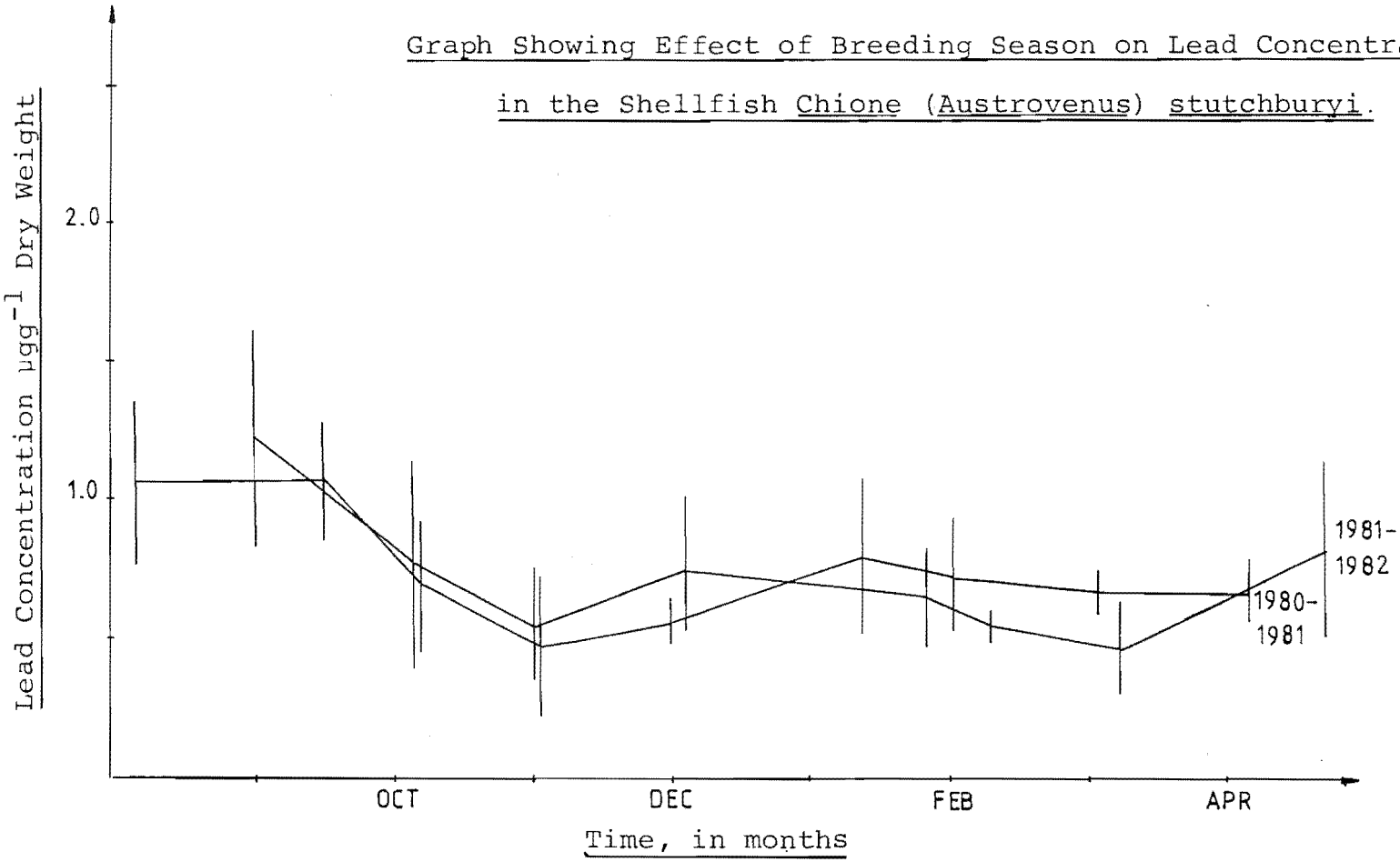


Table 2.4

Effect of Group Weight on Concentration for Seasonal Effect Study.

	Year			
	1980-81		1981-82	
Month	Average Concentration ($\mu gPbg^{-1}$)	Average Dry Weight (g)	Average Concentration ($\mu gPbg^{-1}$)	Average Dry Weight (g)
August	1.06±.31	.55±.15		
September	1.07±.21	.47±.13	1.23±.41	.51±.13
October	.69±.23	.51±.17	.77±.38	.47±.14
November	.48±.26	.51±.10	.55±.21	.63±.11
December	.57±.08	.52±.13	.76±.25	.55±.15
January	.80±.27	.63±.19	.65±.19	.47±.10
February	.72±.21	.57±.16	.54±.08	.60±.10
March	.68±.07	.49±.20	.47±.18	.49±.09
April	.67±.07	.57±.10	.83±.32	.70±.13

Table 2.4 cont.

Notes: (1) Each value is an average of 10 individual species.

(2) Regression Analysis: Average concentration=0.84-0.18×(average dry weight). r =.003

(3) Values are mean ± standard deviation.

2.3.4 Analysis of Shellfish Parts.

The investigation of particular organs within shellfish has been carried out previously with two main aims. The first is to gain information on how heavy metals are accumulated and the second is an attempt to explain the seasonal sexual based changes as discussed above in Section 2.3.3, in terms of changes of concentrations within specific organs of the organism.

In this study investigation of animal parts was carried out to investigate the distribution of lead within the indicator organism, and knowing this and having some idea of the physiological changes that occur within the shellfish, obtain some idea as to how the organism physiology influences the levels of lead in the shellfish.

The shellfish were divided into five main groups of organs (Figure 2.8):

(i) Mantle and Syphons.

(ii) Gills, two, from each side of the body.

(iii) Foot muscle.

(iv) The two adductor muscles.

(v) The stomach, digestive organs and in summer the gonad sack.

Figure 2.8

View of the Internal Organs of Chione (Austrovenus) stutchburyi.

Lower Scale in cm

Table 2.5

Lead Concentration in Shellfish Parts. ($\mu g\ Pbg^{-1}$ Dry Weight)

Organs	Summer	Winter
Syphons and Mantle	2.7±.4	.47±.02
Gills	3.9±.7	.31±.04
Foot Muscle	5.2±.4	.38±.06
Adductor Muscle	3.0±.3	.31±.01
Stomach and Digestive Organs (and Gonad)	4.9±.2	.68±.20

Notes:

Summer values are an average over 10 shellfish (December 1979)

Winter values are an average over 5 shellfish (August 1982)

From the results presented in Table 2.5 the following observations may be made. In winter the organs with the distinctly highest lead level are those of the digestive system and stomach, while in summer this group of organs does not have the highest value. This may be accounted for by the production of gonad, which surrounds the digestive system, in the summer months. As it was not possible to consistently separate the gonad from the rest of this group of organs it was left as one group and values for the lead concentration of gonad have to be obtained by implication. However, other authors have measured lead levels in gonad of various shellfish and found the concentration of lead in gonad low compared to that of the digestive system (6, 15, 16, 22, 33-36).

In this study it was found that the stomach and digestive system had the highest concentrations of lead. Several studies on mussels have shown that the highest concentrations occur within the kidney with the digestive glands next highest (6, 15). Unfortunately in this study it was not possible to consistently isolate the kidney.

In experiments where mussels lived in water to which lead had been added it was found that the gills of the shellfish were the organ whose lead concentration rose most rapidly, and that when removed from these solutions and placed back into their natural environment the lead levels dropped back (16, 34) fairly rapidly. This observation may explain the relatively high levels of lead in gills of Chione during the summer months (Table 2.5), as these shellfish were obtained approximately one month after a peak in lead input to the system.

2.3.5 Use of Chione (Austrovenus) stutchburyi as an Indicator of Lead Levels in the Avon-Heathcote Estuary over Time.

The aim of this study was to look at the variance in lead levels within the Avon-Heathcote Estuary over a period of time, to see if the input of lead was constant and to try to identify its possible sources. The study was carried out by the analysis of batches of 10 cockles (of similar size) collected each month over the period of time from November 1978 to December 1982.

For this work a single sampling site was chosen. The site was approximately 250 m northwest of the first corner

on Beachville Road. (See Figure 2.5). The site was chosen for the following reasons:

(i) Its distance from major vehicular routes, hence minimising contamination from air-borne lead.

(ii) As it is on a major branch of the river, even at low tide it would be representative of river borne lead levels.

(iii) The site is conducive to Chione growth, hence animals reach a large size and their population is large.

(iv) It was possible at low tide to have access to the site by foot.

The results of this study are presented in Table 2.6 and Figure 2.9. The data on the graph illustrates two main features, firstly, a steady decline in average lead concentrations in shellfish over the four year period. The second notable feature is two large peaks in lead concentration, one just after December 1978 and the second just after January 1981. These dates correspond to two major rainfall effects, viz. December 1978 was the wettest December since 1943, and major flooding resulted from a storm of major proportions on 2nd January 1981. This suggests that some of the variance in lead concentrations is due to rainfall. Rainfall data was obtained from the Christchurch office of the New Zealand Meteorological Service viz. total monthly rainfall, peak daily rainfall and peak hourly rainfall rate (37). This data is presented in Table 2.7 and is plotted together with the lead levels in Chione in Figures 2.10-2.12. It would appear that the most likely

Table 2.6

Lead Concentration in Chione (Austrovenus) stutchburyi as a Function of Time (in $\mu gPbg^{-1}$ dry weight).

Year	January	February	March	April	May	June
1978						
1979		4.9±2.8 (19) 5.6±3.5 (23)	5.7±1.6 (7)		3.0±2.6 (15)	.51±.16 (17)
1980	2.0±0.7 (15)	3.7±1.7 (4) 1.7±.6 (11)	1.4±.5 (4)	1.1±.4 (14)	1.23±.32 (13)	.92±.26 (3)
1981	.80±.27 (13)	.72±.21 (2)	.68±.07 (2)	.67±.11 (4) .58±.10 (16)	.42±.10 (6)	.64±.31 (8)
1982	.65±.19 (26)	.54±.08 (2)	.47±.18 (8)	.83±.32 (21)	.53±.15 (15)	.56±.29 (4)

Table 2.6 cont.

Year	July	August	September	October	November	December
1978					1.5±.4 (25)	2.4±.8 (5)
1979	1.71±.63 (10)	.98±.34 (6) .72±.21 (13) .59±.1 (20)	.44±.06 (3)	1.02±.71 (24)	1.02±.73 (12)	1.36±.52 (5)
1980	1.28±.43 (7)	1.06±.31 (4)	1.07±.21 (15)	.69±.23 (6)	.48±.26 (3)	.57±.08 (1)
1981	.80±.13 (14)	.69±.21 (1)	1.23±.41 (1)	.77±.38 (5)	.55±.21 (2)	.76±.25 (3)
1982	.60±.11 (5)	.42±.10 (9)	.78±.16 (2)	.57±.31 (4)	.76±.16 (1)	.85±.09 (2)

Note: (1) Data in table of the form, mean standard deviation of 10 results. Figure in brackets is the day of collection.

Figure 2.9

Graph Showing the Variation in Lead Concentration in Chione (Austrovenus) stutchburyi over a Period of Time in the Avon-Heathcote Estuary, Christchurch.

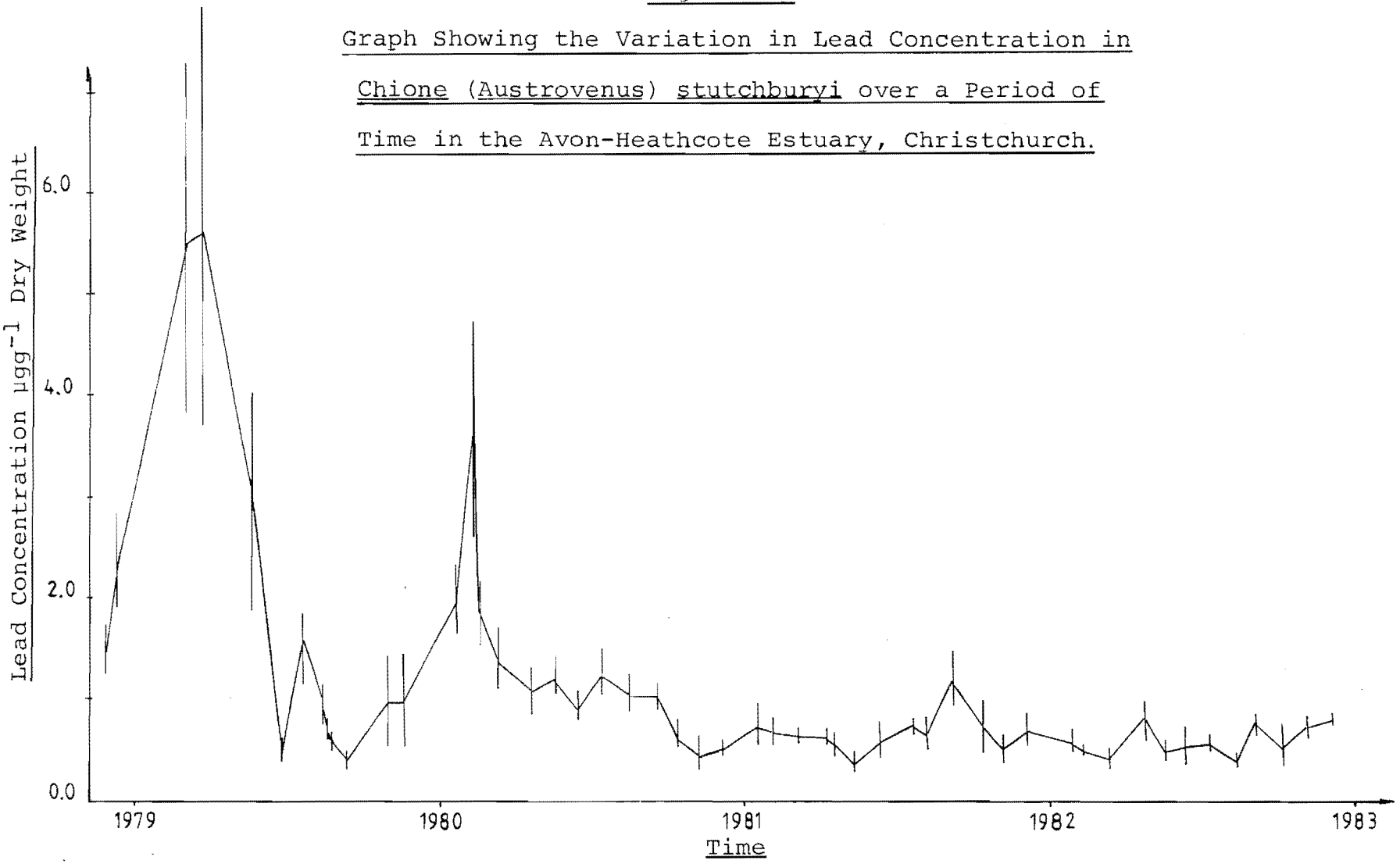


Table 2.7

Rainfall Data for Christchurch, New Zealand.

Year	January	February	March	April	May	June
1978						
1979	8.7	48.5	173.4	11.0	101.8	5.2
	4.3 (4)	15.5 (22)	53.3 (21)	2.9 (30)	32.3 (5)	3.5 (30)
	1.0	3.2	12.0	2.2	3.1	0.9
1980	138.9	41.9	128.6	82.6	16.0	76.0
	110.4 (2)	7.8 (14)	48.9 (2)	28.9 (28)	8.8 (25)	38.8 (4)
	22.4	2.7	5.0	4.8	6.5	4.8
1981	13.8	9.7	46.5	40.5	28.6	113.9
	7.6 (20)	3.1 (23)	13.2 (27)	19.7 (14)	14.3 (19)	33.6 (12)
	2.7	1.6	3.8	3.8	3.0	7.0
1982	12.3	24.2	12.1	50.7	26.4	23.5
	4.3 (7)	21.0 (23)	3.2 (31)	16.7 (4)	9.5 (1)	4.7 (25)
	4.9	4.9	1.5	1.9	1.4	2.2

Table 2.7 cont.

Year	July	August	September	October	November	December
1978			87.3	47.0	35.5	148.7
			30.9 (15)	23.0 (20)	14.0 (15)	38.2 (21)
			5.9	2.9	3.9	30.5
1979	83.8	81.5	17.9	106.6	96.5	25.7
	26.8 (14)	21.9 (1)	4.9 (18)	25.6 (14)	45.3 (20)	10.1 (27)
	4.5	4.3	1.3	5.5	17.6	2.7
1980	32.9	35.7	1.5	11.7	65.1	18.4
	14.5 (15)	12.3 (2)	0.9 (9)	3.1 (15)	12.5 (7)	8.0 (8)
	2.4	3.8	0.9	1.3	2.4	2.3
1981	58.0	100.8	15.4	71.3	36.4	16.7
	22.4 (5)	36.7 (26)	6.6 (20)	20.3 (5)	9.3 (8)	5.1 (23)
	5.9	5.4	1.2	4.9	2.2	2.9
1982	53.3	14.3	20.7	76.3	38.3	63.2
	25.3 (19)	6.6 (17)	7.1 (8)	20.8 (26)	10.5 (12)	13.3 (29)
	5.3	4.9	1.6	6.8	3.9	4.9

Table 2.7 cont.

Notes: (1) Data in the form : Total Monthly Rainfall given in mm.

Highest Daily Rainfall given in mm (day this occurred).

Peak Rainfall Rate given in $mmhr^{-1}$.

Figure 2.10

Graph Showing Total Monthly Rainfall and Lead Concentration in Chione (Austrovenus) stutchburyi in the Avon-Heathcote Estuary as a Function of Time.

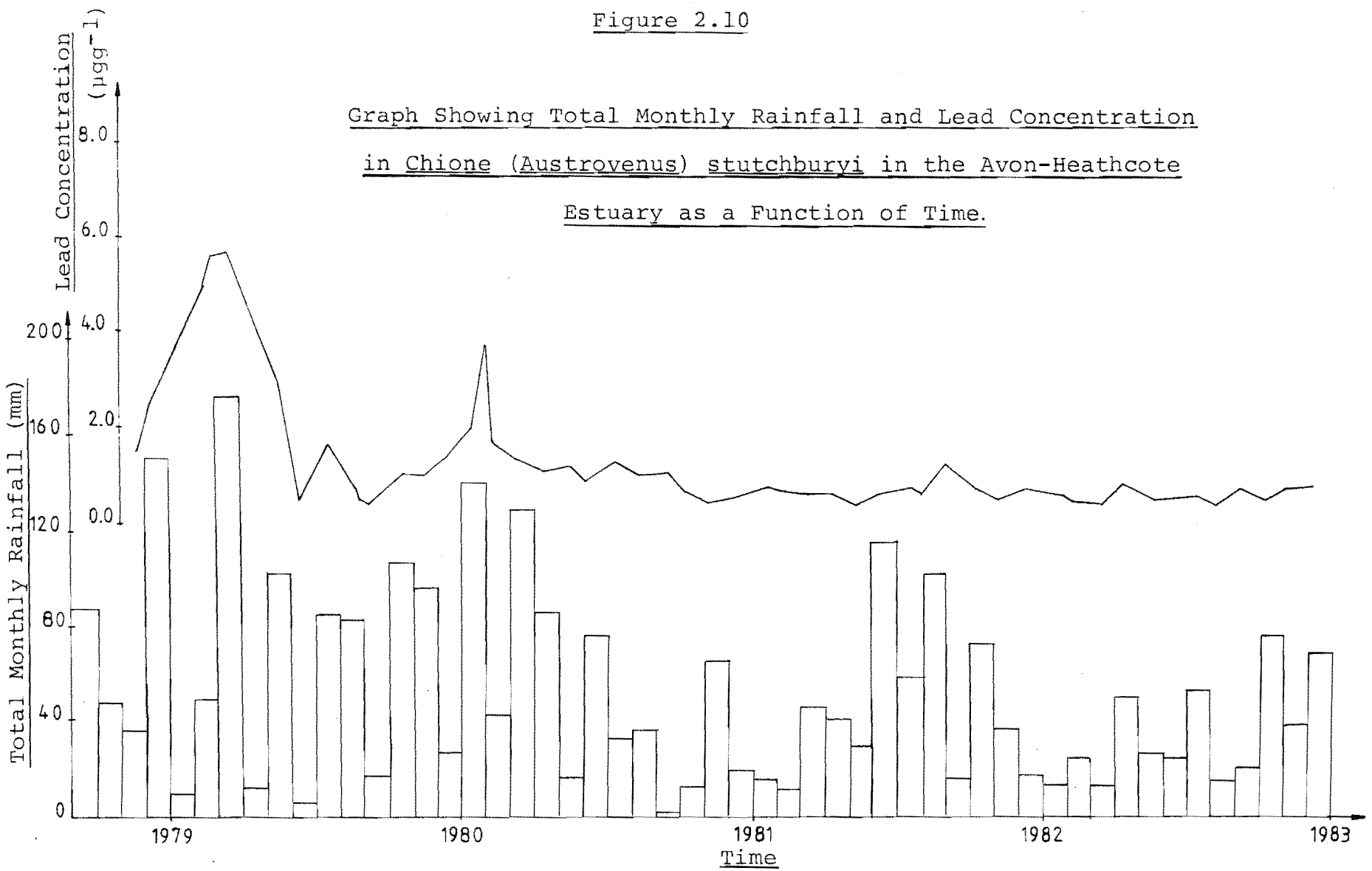


Figure 2.11

Graph Showing Lead Concentration in Chione (Austrovenus) stutchburyi and the Highest Daily Rainfall Per Month as a Function of Time.

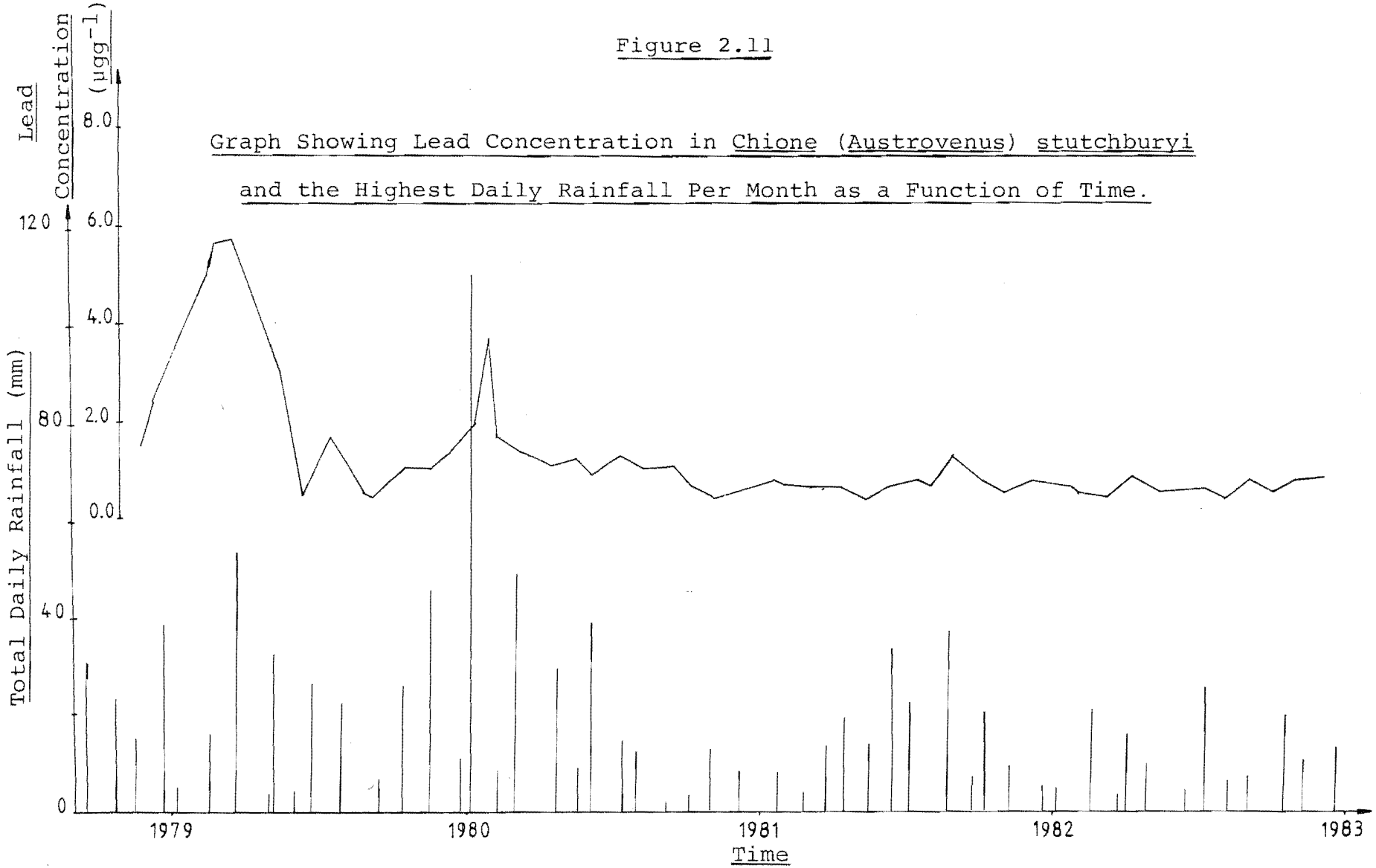
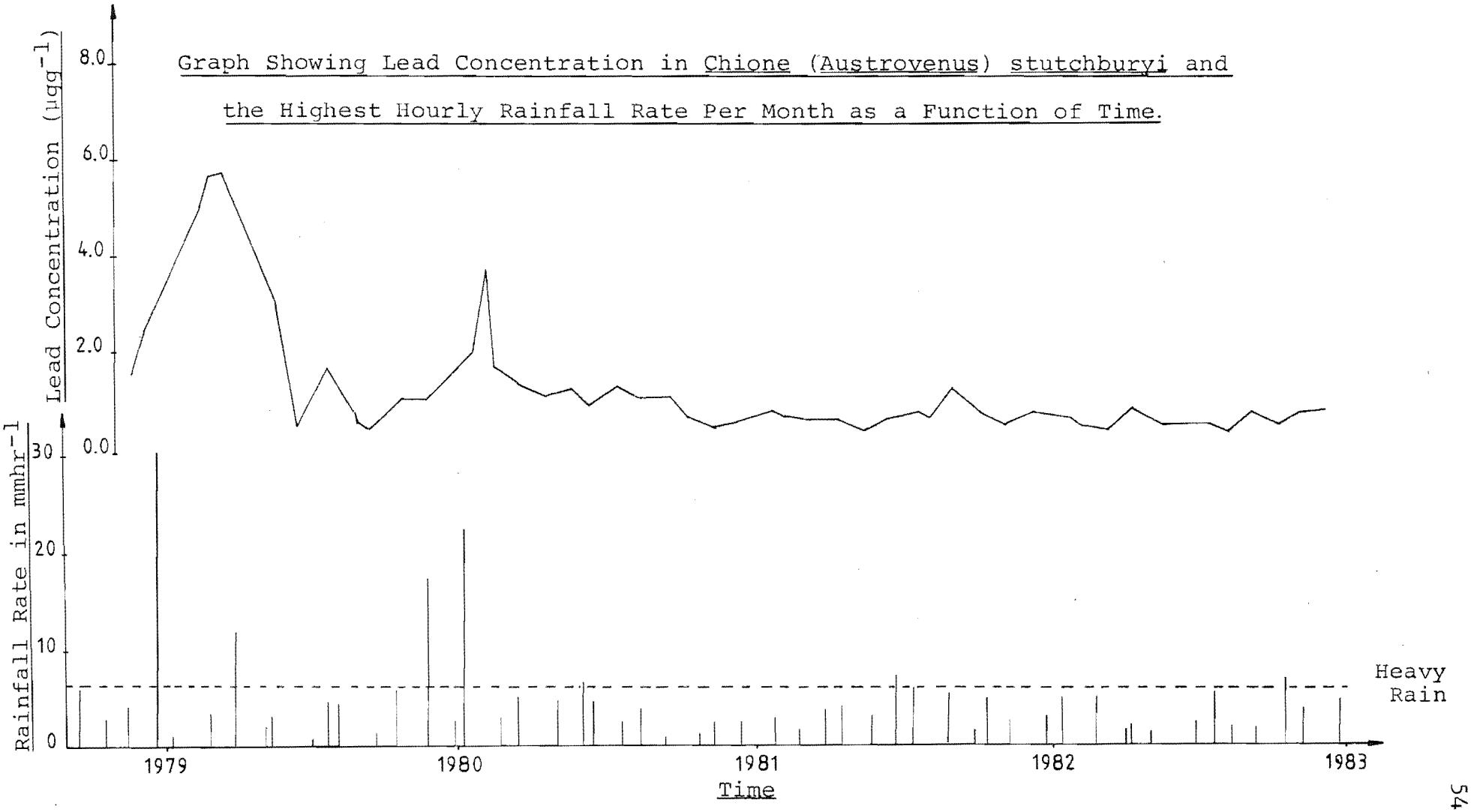


Figure 2.12

Graph Showing Lead Concentration in Chione (Austrovenus) stutchburyi and the Highest Hourly Rainfall Rate Per Month as a Function of Time.



rainfall function which influences the shellfish lead levels is that of peak hourly rainfall rate. (See Figure 2.12). It also appears that the peak load concentrations occur one or two months behind the high rainfall rates, and then return to background levels by four to six months. A rainfall rate greater than 6 mm hr^{-1} is classified as heavy rain and rain falling at this rate (or greater) for a period of hours produces surface flooding in Christchurch. Also, on the occasions of December 1978 and January 1981 the rainfall was sufficient to cause the Heathcote river to break its banks.

The decline of lead concentrations over the four years allows an inference as to the source of lead for the shellfish to be drawn. As the level of lead additions in petrol has not decreased, and the use of cars has not diminished, it would appear reasonable to believe that the major source of lead for shellfish in the estuary is from industrial disposal of waste. Evidence for this conclusion is given in Chapter 4, Section 4.3. Another factor which influences the system, is that the Avon-Heathcote Estuary is deepening due to the increased run off from the Christchurch city area (38). This means that heavy metals are not accumulating in the estuary, in fact the estuary is self flushing of sediment.

2.3.6 Geographical Variation of Lead Concentration with the Avon-Heathcote Estuary as Indicated by Chione (Austrovenus) stutchburyi.

Only limited work was carried out on geographical

variation of lead concentration in cockles. The site used for the time study was used as a reference site (see Section 2.3.5). The other three sites considered were:

(i) A site in Monck's Bay, approximately 25 m toward the estuary outlet from the boat ramp of the Christchurch Yacht Club. This site was chosen to see if there was any significant fall off in lead levels towards the estuary mouth.

(ii) A site near the outflow of McCormack's Bay just past the junction of the causeway and Beachville Road near the road. This was to see if road traffic had any significant effect on the lead levels of the shellfish.

(iii) A site in McCormack's Bay to see if this area was different from the rest of the estuary as it has only partial tidal exchange due to the causeway. (refer to Figure 2.5).

The results of this study are given in Table 2.8. The only significant difference occurs between site (iii) (McCormack's Bay) and the others. However, as explained in Section 2.3.2 this may not be as significant as it first appears.

Table 2.8

Variance in Lead Concentration in Chione (Austrovenus) stutchburyi with Geographical Placement within the Avon-Heathcote Estuary.

	Site (i)	Site (ii)	Site (iii)
Lead Concentration in μgg^{-1} Dry Weight	.77±.27 (7)	4.9±2.8 (10)	.59±.17 (10)
Time of Analysis	August 1979	February 1979	June 1983
Concentration at Reference Site for Comparison with above site	.72±.21 (7)	5.7±1.6 (10)	.30±.10 (10)
Z-statistic for $H_o: X_1 = X_2$ from t-test	.39	-.40	4.53
Level of Significance	N.S.	N.S.	.005

Notes:- (1) N.S. means not significant.

(2) The t-test is for the null hypothesis that there is no difference between a site and the reference site.

(3) Figure in brackets is the number in the sample.

(4) Values are mean±standard deviation.

Table 2.9

Cadmium Concentration in Chione (Austrovenus) stutchburyi from the Avon-Heathcote Estuary, Christchurch, New Zealand

		Concentration
Lead		1.4 ± 0.5 µg Pbg^{-1} dry weight
Cadmium		$0.12 \pm .04$ µg Cdg^{-1} dry weight
	or	124 ± 35 ng Cdg^{-1} dry weight

2.3.7 Cadmium Levels in Chione (Austrovenus) stutchburyi in the Avon-Heathcote Estuary.

Only one group of shellfish was analysed for Cadmium as the levels found were very low and not very significant. These results were obtained for March 1980, and the lead levels are given in comparison (see Table 2.9). The site used was the same as for the time study of lead in cockles (see Section 2.3.5 and Figure 2.5).

2.3.8 Comparisons of these Results with other Results Obtained with Chione (Austrovenus) stutchburyi.

As noted earlier this shellfish has not been widely used as an indicator of heavy metal pollution. A table listing comparative data on lead and cadmium levels in cockles is given in Table 2.10.

The reported results were obtained using flame atomic absorption spectrophotometry, except those of Chow et al (39) who used mass spectrometry. In two cases batched samples

Table 2.10

Concentration of Lead and Cadmium in the Cockle Chione (Austrovenus) stutchburyi.

Location	Pb Range	Mean	Cd Range	Mean	Time	Reference
Wairoa Bay, Bay of Islands, N.Z.[1]		1.8		0.19	1975	Nielsen & Nathan (11)
Waikawa, Bay, Marlborough Sounds	BDL-53	13.2		<0.015	1977	Stephenson (12)
McCormacks Bay, Christchurch		52		1.4	1975	Millhouse (9)
Avon-Heathcote Estuary, Christchurch	BDL-33	14	BDL-10.2	3.4	1977	Millhouse (10)
Avon-Heathcote Estuary, Christchurch	1.1-10.8	3.1	0.25-1.7	0.8	1979	Drainage Board (13)
Brooklands Lagoon, Christchurch		3.8		0.7	1979	Drainage Board (13)
Avon-Heathcote Estuary, Christchurch	0.30-5.7	1.2	0.09-0.18	0.12	1979-83	This Work
Bahia de Todos Santos Baja, California[2]	0.18-0.6	0.35			1973	Chow et al. (40)

Notes: (1) These results are given as wet weight. From this work it was found that the dry weight is approximately 20% of the wet weight, hence values should be multiplied by approximately 5 times.

(2) These shellfish were not Chione (Austrovenus) stutchburyi but were listed merely as Chione sp.

Table 2.10 cont.

Notes cont.: (3) BDL means below detection limit.

(4) Cd range is in $\mu g Cd g^{-1}$.

(5) Pb range is in $\mu g Pb g^{-1}$.

of 14 (11) and 10 (13) cockles were used to obtain detectable sample sizes. Both Stephenson (12) and Millhouse (9, 10) state that in some of their samples the metal levels were below their detection limits (neither mentioned the limit). In calculating their respective mean concentrations the authors state they used the detection limit.

The results obtained in this work are in line with those obtained by the Christchurch Drainage Board (13) in a preliminary report. Although levels of lead have dropped within the Avon-Heathcote Estuary it appears highly unlikely that they have dropped sufficiently for the results of Millhouse (9, 10) to be in line with this study. There is insufficient data to draw a conclusion on whether levels of lead are high in the Avon-Heathcote Estuary compared with other sites within New Zealand but it would appear that they are not.

2.4.1 Evaluation of Chione (Austrovenus) stutchburyi as Bio-Indicator.

The conclusion of this study is that Chione does accumulate lead and cadmium. The shellfish acts as short term indicator integrating levels over approximately two-three month periods. However, it was not established if the levels of lead or cadmium from Chione varied in a linear way with the lead and cadmium in the estuary. The results of the study also revealed that lead concentrations in Chione do depend on the size of the shellfish and whether or not the shellfish is undergoing either maturation or spawning.

Lead was concentrated within the digestive organs of the shellfish. This result is supported by Stephenson (39) who reported that Chione is an indiscriminant filter-feeder, feeding on particulate organic matter of terrestrial, marine and estuarine origin depending upon its position within the estuary. As lead would appear to be obtained from particulate matter brought down by the rivers, this would account for the high concentration of lead within the digestive system.

No effect could be put down to salinity or height within the water column, although Phillips (4) noted these affected the indicator ability of Mytilis edulis. Explanation for this may be that as the system has almost complete tidal exchange, Chione suffers daily salinity changes and hence integrates over them. Chione being a sediment dwelling bivalve, living in an intertidal zone has various heights of water column above it. The shellfish appear to tolerate this, but if exposed for too long they will not grow to maximum size (41).

2.4.2 Suggested Procedure for the use of Chione (Austrovenus) stutchburyi as a Bio-Indicator.

The cockle can be used as a bio-indicator providing the following points are noted:

(i) The sample should be made up of large, sexually mature specimens of similar weight. This is to minimise the problems of size/concentration variation. If this is not possible, then specimens should be of a large

range of sizes to allow for size/concentration regressions to be made.

(ii) If geographical surveys are planned using Chione as a bio-indicator then this is best done in the period May-August when the shellfish are dormant (32). This avoids the problems of maturation and spawning affecting the concentrations.

(iii) If time variation studies are planned then the effects of maturation and spawning producing a seasonal variation must be considered.

References.

1. Pytkowicz, R.M.; Kester, D.R. The physical chemistry of sea water. Oceanogr. Mar. Biol. Annu. Rev., 9, 11-60, (1971).

2. Butler, P.A.; Andrén, L; Bonde, G.J.; Jernelöv, A; Reish, D.J. Monitoring organisms. In: FAO Technical Conference on marine pollution and its effects on living resources and fishing, Rome, 1970. Suppl. 1: Report of the seminar on methods of detection, measurement and monitoring of pollutants in the marine environment, pp 101-112, FAO Fisheries Reports No. 99, Suppl. 1, (1971).

3. Haug, A; Melsom, S; Omang, S. Estimation of heavy metal pollution in two Norwegian fjord areas by analysis of the brown agla Ascophyllum nodosum. Environ. Pollut., 7, 179-192, (1974).

4. Phillips, D.J.H. The common mussel Mytilus edulis as an indicator of pollution by zinc, cadmium, lead and copper. I. Effects of environmental variables on uptake of metals. Mar. Biol., 38, 59-69, (1976).

5. Phillips, D.J.H. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments - a review. Environ. Pollut., 13, 281-317, (1977).

6. Manly, R; George, W.O. The occurrence of some heavy metals in populations of the freshwater mussel Anodonta anatina (L.) from the River Thames. Environ. Pollut., 14, 139-154, (1977).

7. Julshamn, Kaare. Studies on major and minor elements in molluscs in Western Norway. III. Effects of size and age on the contents of 10 elements in oyster (Ostrea edulis). taken from unpolluted waters. Fiskeridir. Skr., Ser. Ernaer., 1, 199-214, (1981).

8. Boyden, C.R. Effect of size upon metal content of shellfish. J. Mar. Biol. Assoc. (U.K.), 57, 675-714, (1977).

9. Millhouse, D. The trace metal content of animals from McCormacks Bay, Avon-Heathcote Estuary, Christchurch. Estuarine Research Unit Report No. 4, Dec. 1975. Estuarine Research Unit, Dept. of Zoology, Univ. of Canterbury, Christchurch, New Zealand.

10. Millhouse, D. Trace metals in the Avon-Heathcote Estuary, Christchurch, New Zealand. Estuarine Research Unit Report No. 8, June 1977. Estuarine Research Unit, Dept. of Zoology, Univ. of Canterbury, Christchurch, New Zealand.

11. Nielsen, S.A.; Nathan, A. Heavy metal levels in New Zealand molluscs. N.Z. J. Mar. Freshwater Res., 9, 467-481, (1975).

12. Stephenson, R.L. Waikawa bay: intertidal biological survey. Prepared for Marlborough Harbour Board, July 1977.

13. Private communication of preliminary results, from Christchurch Drainage Board.

14. Stephenson, R.L. Aspects of the energetics of the cockle Chione (Austrovenus) stutchburyi in the Avon-Heathcote Estuary, Christchurch, New Zealand. PhD.

Thesis in Zoology, Univ. of Canterbury, pp 165, (1981)

15. Schulz-Baldes, M. Die Miesmuschel Mytilus edulis als Indikator fur die Bleikonzentration im Weserästuar und in der Deutschen Bucht. Mar. Biol., 21, 98-102, (1973).

16. Schulz-Baldes, M. Lead uptake from sea water and food, and lead loss in the common mussel Mytilus edulis. Mar. Biol., 25, 177-193, (1974).

17. Phillips, D.J.H. The common mussel Mytilus edulis as an indicator of pollution by zinc, cadmium, lead and copper. II. Relationship of metals in the mussel to those discharged by industry. Mar. Biol., 38, 71-80, (1976).

18. Boalch, R; Chan, S; Taylor, D. Seasonal variation in the trace metal content of Mytilus edulis. Mar. Pollut. Bull., 12, 276-280, (1981).

19. Bryan, G.W.; Hummerstone, L.G. Heavy metals in the burrowing bivalve Scrobicularia plana from contaminated and uncontaminated estuaries. J. Mar. Biol. Assoc. (U.K.), 58, 401-419, (1978).

20. Mackay, N.J; Williams, R.J.; Kacprzac, J.L.; Kazacos, M.N; Collins, A.J.; Auty, E.H. Heavy metals in cultivated oysters (Crassostrea commercialis=Saccostrea cucullata) from the estuaries of New South Wales. Aust. J. Mar. Freshwater Res., 26, 31-46, (1975).

21. Ayling, G.M. Uptake of cadmium, zinc, copper, lead and chromium in the pacific oyster, Crassostrea gigas, grown in the Tamar River, Tasmania. Water Res., 8, 729-738, (1974).

22. Chow, T.J.; Snyder, H.G.; Snyder C.B. Mussels (Mytilus

sp.) as an indicator of lead pollution. Sci. Total Environ., 6, 55-63, (1976).

23. Harvey, E.J. Sr; Knight, L.A. Jr. Concentration of three toxic metals in oysters (Crassostrea virginica) of Biloxi and Pascagoula, Mississippi estuaries. Water, Air Soil Pollut., 9, 255-261, (1978).

24. Orren, M.J.; Eagle, G.A.; Hennig, H.F.-K.O; Green, A. Variations in trace metal content of the mussel Choromytilus meridionalis (Kr.) with season and sex. Mar. Pollut. Bull., 11, 253-257, (1980).

25. Latouche, Y.D.; Mix, M.C. Seasonal variation in soft tissue weights and trace metal burdens in the bay mussel, Mytilus edulis. Bull. Environm. Contam. Toxicol., 27, 821-828, (1981).

26. Latouche, Y.D.; Mix, M.C. The effects of depuration, size and sex on trace metal levels in bay mussels. Mar. Pollut. Bull., 13, 27-29, (1982).

27. Simpson, R.D. Uptake and loss of zinc and lead by mussels (Mytilus edulis) and relationships with body weight and reproductive cycle. Mar. Pollut. Bull., 10, 74-78, (1979).

28. Majori, L; Nedoclan, G; Modonuti, G.B; Daris, F. Study of the seasonal variations of some trace elements in the tissues of Mytilus galloprovincialis taken in the Gulf of Trieste. Rev. Int. Océanogr. Med., 49, 37-40, (1978).

29. Hsu, Shun-Yao; Wang, Guoo-Shyng; Jeng, Sen-Shyong. The occurrence and seasonal variations of sodium, potassium, calcium, magnesium and heavy metals in Taiwan's oysters and clams. Bull. Inst. Zool., Acad. Sin., 18, 11-20, (1979).

30. Julshamn, Kaare. Studies on major and minor elements in molluscs in Western Norway. II. Seasonal variations in the contents of 10 elements in oysters (Ostrea edulis) from three oyster farms. Fiskeridir Skr., Ser. Ernaer., 1, 183-197, (1981).

31. Kumagai, Hiroshi; Saeki, Kiyoko. Seasonal variation in heavy metal content of the edible portion of short-neck clam, Tapes japonica. Nippon Suisan Gakkaishi, 46, 851-854, (1980).

32. Larcombe, M.F. The ecology, population dynamics, and energetics of some soft shore molluscs. PhD. Thesis in Zoology, Univ. of Auckland, pp 250, (1971).

33. Young, D.R; Alexander G.V; McDermott-Ehrlich, D. Vessel-related contamination of Southern California Harbours by copper and other metals. Mar. Pollut. Bull., 10, 50-56, (1979).

34. Sivalingam, P.M; Bhaskaran, B. Experimental insight of trace metal environmental pollution problems in mussel farming. Aquaculture, 20, 291-303, (1980).

35. Julshamn, Kaare. Studies on major and minor elements in Western Norway. IV. The distribution of 17 elements in different tissues of oyster (Ostrea edulis), common mussel (Mytilus edulis), horse mussel (Modiolus modiolus) taken from unpolluted waters. Fiskeridir. Skr., Ser. Ernaer., 1, 215-234, (1981).

36. Topping, G. Heavy metals in shellfish from Scottish waters. Aquaculture, 1, 379-384, (1973).

37. Personal communication from Christchurch office, New Zealand Meteorological Service.

38. Macpherson, J.M. Response to urbanization of the Avon-Heathcote Estuary, Christchurch, New Zealand. Environ. Geol., 3, 23-27, (1979).

39. Stephenson, R.L. A stable carbon isotope study of Chione (Austrovenus) stutchburyi and its food sources in the Avon-Heathcote Estuary. Estuarine Research Report No. 22, Sept. 1980, Dept. of Zoology, Univ. of Canterbury, New Zealand, pp 48.

40. Chow, T.J; Snyder, C.B; Snyder, H.G; Earl, J.L. Lead content of some marine organisms. J. Environ. Sci. Heath - Environ. Sci. Eng., A11, 33-44, (1976).

41. Stephenson, R.L. Factors influencing production of the cockle Chione stutchburyi in the Avon-Heathcote estuary, Christchurch, New Zealand. J. Malakol. Soc. Aus., 4, 262, (1980).

Chapter 3

The Analysis and Distribution of some Heavy Metals in the Shells of Chione (Austrovenus) stutchburyi.

3.1.1 Introduction

Although bivalve mollusc bodies (soft parts) have been widely used as indicators of heavy metals, the bivalve shells have not been widely used. Initially, this would appear to be unusual, as the shells could be expected to accumulate metals during their growth, which would then remain fixed, giving an indication of long term exposure.

However, several problems occur with the use of shells as indicators of heavy metal pollution. Although bivalves do not suffer the problems of crustaceans, who moult and hence lose their shells, both classes of marine organism have the following problems as far as their use as indicators is concerned:

(i) Heavy metals may be incorporated into shells either by integration into the shell as new shell is produced, or absorbed onto the shell's surface while the shell is exposed to the marine environment. (1, 2).

(ii) If both methods occur then something must be known about the rates of accumulation of heavy metals by both integration and absorption in order to determine their relative importance. (1, 2, 3).

(iii) Also absorption may reflect a different mode of pollution transport than integration. Integrated heavy

metals in shells should reflect the levels of pollutants within the body of the shellfish and hence respond to the same pollution pathway, for example, uptake of metals by the ingestion of plankton containing heavy metals. On the other hand, absorption is more likely to reflect levels of soluble pollutants which may be absorbed onto the shell surface.

(iv) Since in temperate climates most shell growth occurs over only part of the year, the levels of pollutants integrated into shells will reflect the levels of pollutants at the growth season. Therefore, if pollutant delivery is seasonal this may not reflect the true average integrated pollution load.

(v) The rate of accumulation of heavy metals in shells appears to be lower than for soft tissue, hence the trace metal concentration in shells is lower, which may cause analytical problems.

(vi) The distribution of heavy metals is not uniform throughout the shell. (1, 2, 4).

These problems make the interpretation of results obtained by the use of shells suspect, or at best highly difficult. In this study it was intended to look at some of these factors from an analytical point of view.

3.1.2 Intentions and Outline of This Study.

The intention of this study was firstly to develop an analytical method which would allow for the analysis of

small sections of shell for heavy metals, in particular lead. The second aim was to look at the distribution of lead and some other heavy metals within the shell. In this way it may be possible to look at the effect of absorption on the heavy metal distributions within shells.

The shells of the sediment dwelling, filter-feeder, bivalve mollusc Chione (Austrovenus) stutchburyi were used, and these were taken from the Avon-Heathcote Estuary as described in Chapter 2 (Section 2.1.6 and 2.1.7).

3.2.1 Analytical Methods

The technique chosen was atomic absorption spectrophotometry. Initially a flame atomisation technique was employed on whole shell samples but flameless atomisation was later found to be necessary. For small sections of shell a flameless atomisation technique using carbon-cup atomisation was employed.

Prior to analysis, all shells were soaked overnight in a 1% papain, 1% NaCl solution to remove any organic matter clinging to either the external or internal surfaces of the shells. The shells were then gently scrubbed with a hard nylon toothbrush, washed in distilled water, and then dried for 24 hours at 100^{o}C.

3.2.2 Methods for Whole Shell Analysis.

The first method used in an attempt to analyse lead levels in shells, was to dissolve the shell in nitric acid, and then directly aspirate the resultant solution into the

flame. Such a simplistic method was unsatisfactory, as the concentration of calcium (up to 10% W/V) produced excessive background scattering in the flame as well as causing the burner head to frequently block with dried calcium salts.

Because of this, a solvent extraction method was employed. The system chosen was the complexation of lead with ammonium pyrrolidine dithiocarbamate (APDC) and the subsequent extraction into methyl isobutyl ketone (MIBK). In this method, which was adapted from the work of Mackie et al (5) on lead in teeth, the shell was finely ground in an agate pestle and mortar, until a homogeneous powder was produced. One gram of this powder was then dissolved in the minimum of 2.0M nitric acid and then an ammonium citrate/ammonium hydroxide buffer was added until the solution was approximately pH 4.5, using bromophenol blue as an indicator. A solution of 1% APDC followed by MIBK was added to the mixture in a separating funnel. The mixture was then shaken three times at five minute intervals, after which the organic layer was colleted. A further extraction with MIBK was carried out and added to the first. The resultant MIBK solution was evaporated to dryness on a hot plate, and the residue taken up in 0.5M nitric acid.

Aqueous standards to which 5% W/V calcium carbonate was added and treated in the same manner as the shells, were used to check on the recovery of lead by the organic complexation and extraction method. (See Table 3.1).

Standards for the flameless atomisation atomic absorption spectrophotometry were in the range 0.025 - 0.200$\mu g ml^{-1}$ and made up in 0.5M HNO_3. The reagents were of

Table 3.1

Recovery for Extraction Method.

Standard Concentration ($\mu gPbg^{-1}$)	Aqueous Absorbance	Extracted Absorbance	% Recovery
0.025	12	12	100
0.025	17	17	100
0.050	30	24	80
0.050	40	42	105
0.100	50	47	94
0.100	90	85	94
0.200	95	110	116
0.200	175	195	111
		Mean $\pm$ Standard Deviation =	100$\pm$11

analytical or BDH "Aristar" grade and the blank was found to be not detectable above the noise level.

For this work a Varian AA-5 atomic absorption spectrophotometer was used. The instrument settings were the same as in Chapter 2 (Section 2.2.4) and carbon-cup atomisation was used.

3.2.3 Carbon-Cup Atomisation for Analysis of Shell Fragments.

To look at variation of lead concentration within a cross-sectional slice of shell, a slice approximately 3 mm wide was cut from the umbo to the posterior edge (see Figure 3.1) using a diamond tipped, water cooled saw. The central section of this slice was then cut along the growth lines in the shell to produce samples (as a function of time) approximately 0.1 mm thick and weighing approximately 10 mg. A sample of newly formed shell at the posterior edge of the shell was also obtained.

A second approach was to study the variation in metal concentration with position on the shell surface. To facilitate this a dental burr drill was used to remove the exterior surface from selected areas of the shell. Around 10 - 20 mg of powdered sample wereobtained.

For carbon-cup analysis a Varian CRA-63, carbon rod atomiser with carbon cups was employed attached to a Varian AA-1475 atomic absorption spectrophotometer. Settings for these instruments are given in Table 3.2

Figure 3.1

Diagram Showing Cutting of the Shell for Cross Sectional Analysis.

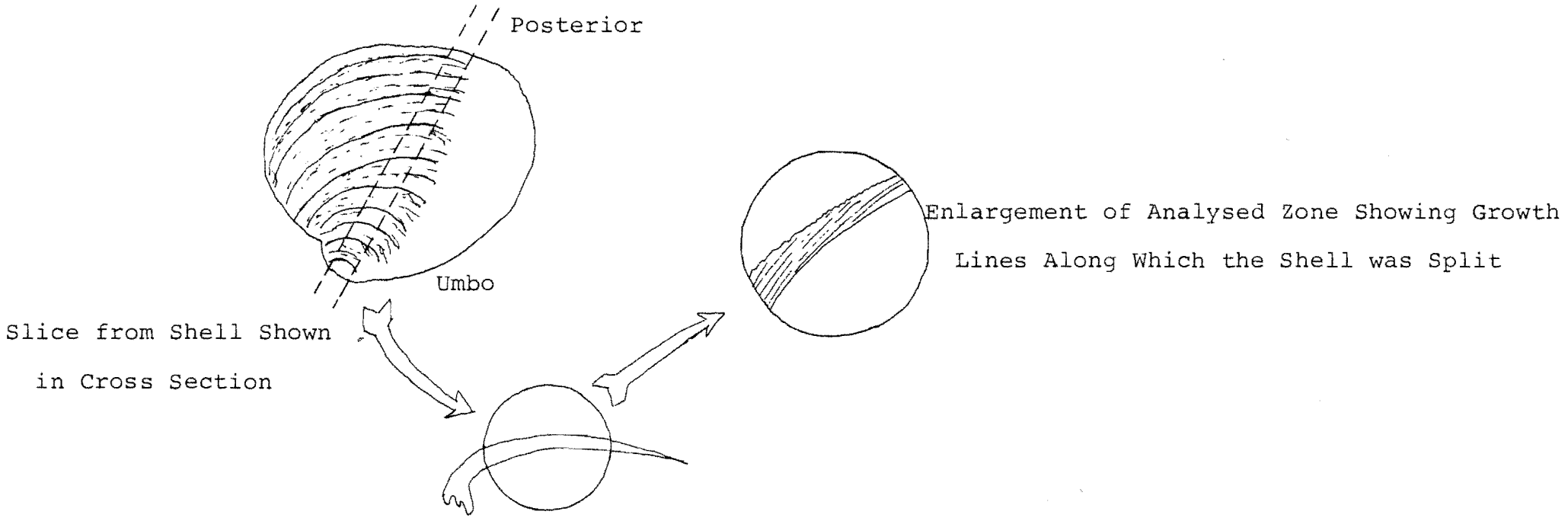


Table 3.2

Settings for Analysis of Shells for Varian AA-1475 Atomic Absorption Spectrophotometer.

Element	Pb	Cu	Cr	Zn
Wavelength (nm)	217.0	324.8	357.9	213.9
Slit Width (nm)	1.0	0.5	0.2	1.0
Mode	Absorbance	Absorbance	Absorbance	Absorbance
Background Corrector	Off	Off	Off	On
Standard Range (μgmL^{-1})	0.025-0.4	0.025-0.4	0.025-0.2	0.40-1.6
Method of Atomisation	Carbon Cup	Carbon Cup	Carbon Cup	Air/Acetylene flame

Settings for Varian CRA-63 Carbon-Rod Atomiser.

Element	Pb	Cu	Cr	Zn
Dry	4.5 (20)	4.5 (20)	4.5 (20)	N.A.
Ash	5.0 (10)	5.0 (10)	5.0 (10)	N.A.
Atomise Mode	Ramp	Ramp	Ramp	N.A.
Cut Off Voltage	7.0	9.0	9.0	N.A.

Table 3.2 cont.

Element	Pb	Cu	Cr	Zn
Ramp Rate	3.0	3.5	4.0	N.A.

Notes: (1) Value in parenthesis is approximately time in seconds.

(2) The ramp mode was used in all carbon-cup analyses so that the calcium peak did not interfere with the analyte element signal.

3.2.4 Checks on the Analytical Methods.

For the carbon rod analyses, a sample of powdered tooth dentine, the lead in which had been previously determined by standard additions and on many replicate samples, was analysed with each analytical run, so that the method and contamination could be checked. The sample was previously analysed 70 times and had a mean lead concentration of $10.3\mu gg^{-1}$ with a standard deviation of $0.1\mu gg^{-1}$ (6). The standard dentine sample was analysed 80 times during this study and the mean lead concentration was $10.3\mu gPbg^{-1}$ with a standard deviation of $0.2\mu gPbg^{-1}$. This was therefore in agreement with the result of Fergusson et al. (6).

To check on the cleaning process, (see Section 3.2.1) a group of shells, which had been cleaned as described, was further cleaned by placing in an ultra-sonic bath for 15 minutes. There was no difference in the lead levels between the two halves of one shell, (one half treated normally, while the other also had ultra-sonic cleaning) except for experimental error. Surface samples of these shells were also analysed for copper, chromium and zinc. The ratio of concentration of elements after ultra-sonic treatment compared with the concentration of the normally treated shell, expressed as a percentage are: lead 89%, chromium 122% copper 133% and zinc 96%. From this, it is concluded that the ultra-sonic cleaning did not significantly lower surface element concentrations, and that the cleaning process employed was satisfactory.

3.3.1 Results and Discussion

It appears that this study is the first carried out on the shells of Chione (Austrovenus) stutchburyi. Therefore the results can only be compared with those obtained on the shells of mussels, oysters, clams and fresh water shellfish.

3.3.2 Results of Whole Shell Analyses.

The results for whole shell analyses are given in Table 3.3. The mean concentration of lead in the shells was $1.17 \mu gPbg^{-1}$ with a standard deviation of $0.59 \mu gPbg^{-1}$. The lead concentration is of the same magnitude as that obtained in whole soft tissue of Chione (Austrovenus) stutchburyi on a dry weight basis. Chow et al (7) reported that for Chione sp, the lead concentration was lower in the shell than in the soft tissue (dry weight). Other authors have found for other species that the shells of molluscs generally have lower lead concentrations than the whole soft tissue on a dry weight basis (4, 6).

When the data on lead concentration in the shells of Chione (Austrovenus) stutchburyi was plotted as a function of the weight of the shell, (see Figure 3.2) no weight/ concentration relationship was observed. This differs from the results of Clarke et al (8) who found a strong weight/ concentration relationship for the fresh water mollusc Corbicula Manillensis. However, this may be because very small shells were not included in the present study, and Clarke et al (8) found that for large shells the concentration

Table 3.3

Lead Concentrations in the Shells of Chione (Austrovenus) stutchburyi.

Shell Weight (g)	Shell Length (mm)	Lead Concentration ($\mu gPbg^{-1}$)
3.75	30	1.3±0.1
4.25	34	1.5±0.2
4.87	N.A.	0.82±0.12
5.03	33	2.7±0.2
5.3	35	0.8±0.1
5.46	33	0.7±0.2
5.83	37	0.9±0.02
6.93	N.A.	1.64±0.07
7.55	38	0.8±0.1
9.74	40	1.0±0.1
9.74	N.A.	1.4±0.6
10.97	N.A.	0.52±0.12

Table 3.3 cont.

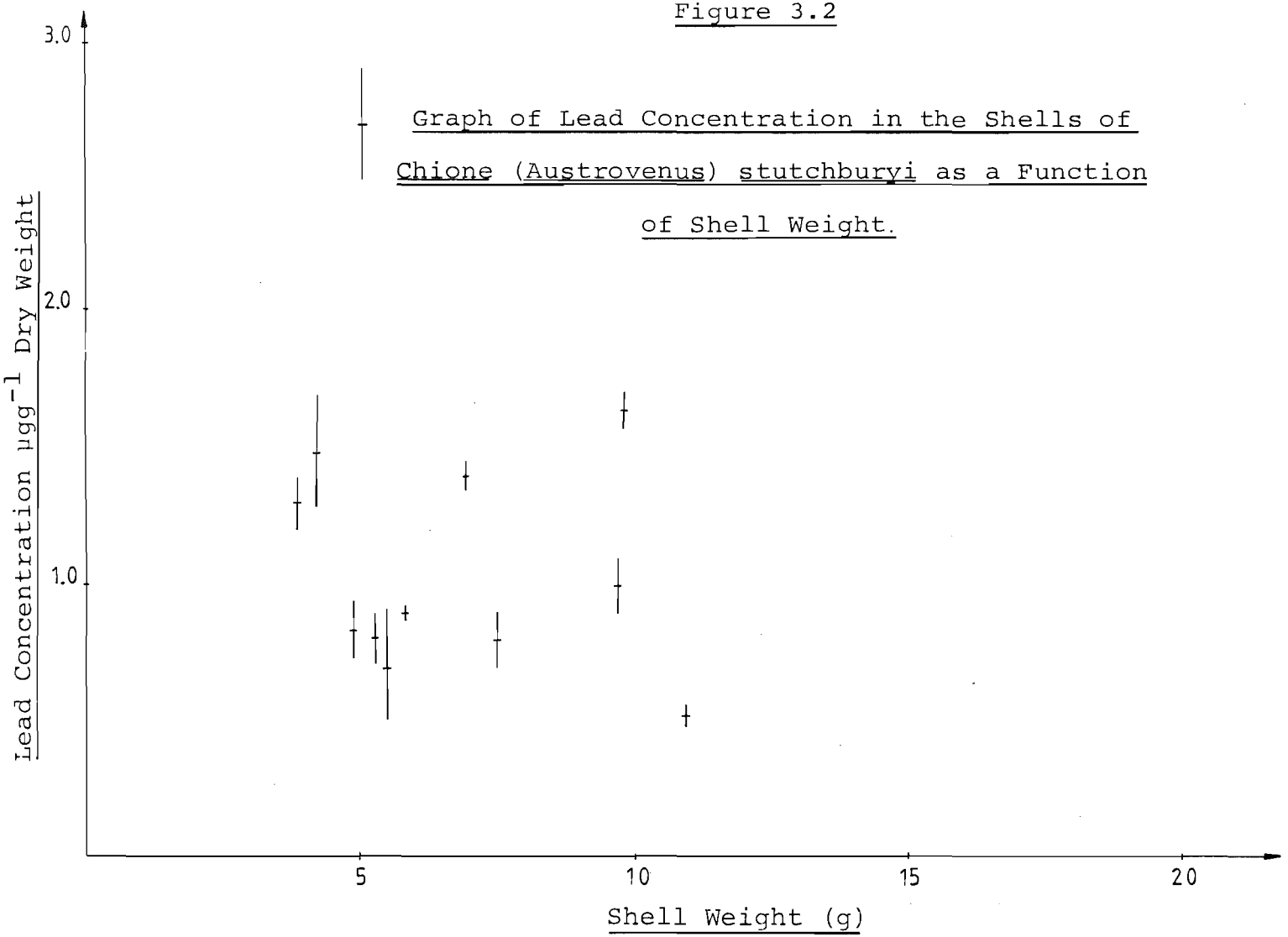
Shell Weight (g)	Shell Length (mm)	Lead Concentration ($\mu gPbg^{-1}$)
6.62 (average)		1.2 (average)
2.38 (standard deviation)		0.6 (standard deviation)

Notes: (1) N.A. means no shell length data was available for these shells.

(2) Values are mean±error.

Figure 3.2

Graph of Lead Concentration in the Shells of Chione (Austrovenus) stutchburyi as a Function of Shell Weight.



of lead was fairly constant.

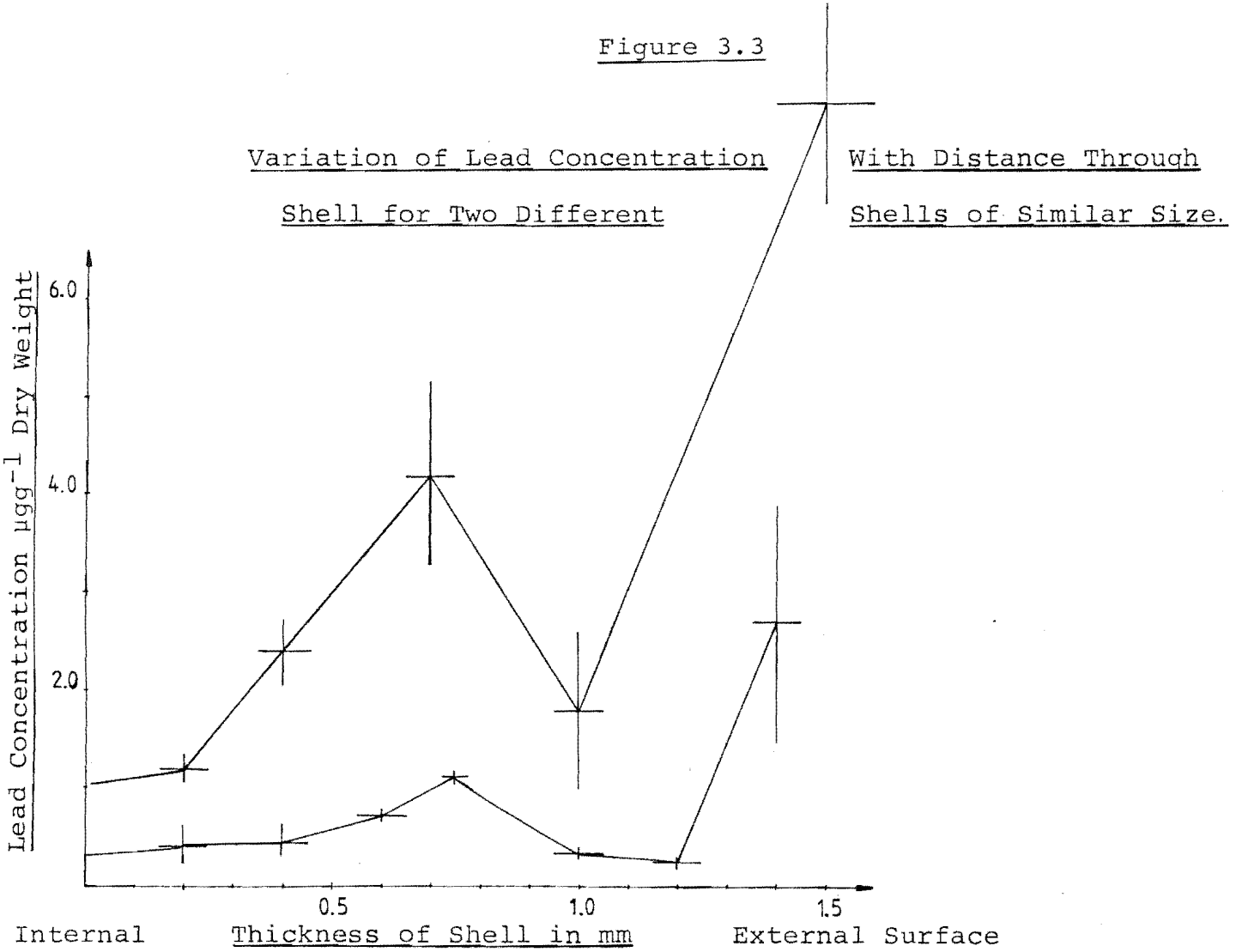
3.3.3 Results of Sectional Analysis of Shells.

As stated in Section 3.2.3 small slices along the growth lines were taken from a cross sectional slice of a shell. It was found that this fragmentation was made easier if the shells were first heated to 400^{o}C for 24 hours. Some thermal decomposition of the organic matrix within the shell occurred allowing for easier division along the growth lines. There was no evidence of lead loss during this process (see Chapter 2 (Section 2.2.2 (b)).

The data as shown on Figure 3.3 illustrates the distribution of lead in a cross section through the shell of two cockles. Three points of interest may be noted. Firstly, the lead concentration in the whole soft tissue of the shellfish was 4.5$\pm$ 1.1μgPbg^{-1} (dry weight), this suggests that there is some discrimination against the incorporation of lead into the shell matrix from the mantle. The second feature is the relatively high levels of lead on the external surface of the shell. The third feature of interest is an apparent rise in lead concentration within the centre of the shell for both samples. As the shell was sliced, approximately along yearly growth lines, it is tempting to suggest that the high level of lead in the centre of the shell is due to a period in the past where higher levels of available lead existed. Though the variation of lead with shell thickness for the two shells studied agree well with each other (see Figure 3.3) further shells should be investigated to verify this.

Figure 3.3

Variation of Lead Concentration With Distance Through Shell for Two Different Shells of Similar Size.



A further investigation of the high lead levels on the external surface of the shells was carried out. The elements cadmium, copper and zinc were also analysed. The shells were divided into either nine sectors (large shell), or four sectors (small shell), see Figure 3.4. The results for lead are given in Table 3.4 and the results for one shell (representative of large shells) are shown in diagrammatic form in Figure 3.5

Three observations can be made from this data:

(i) The concentration of lead is highest in the umbo region of the shell (which is the oldest part of the shell surface), and lowest in the posterior section of the shell (which is the youngest part of the shell surface).

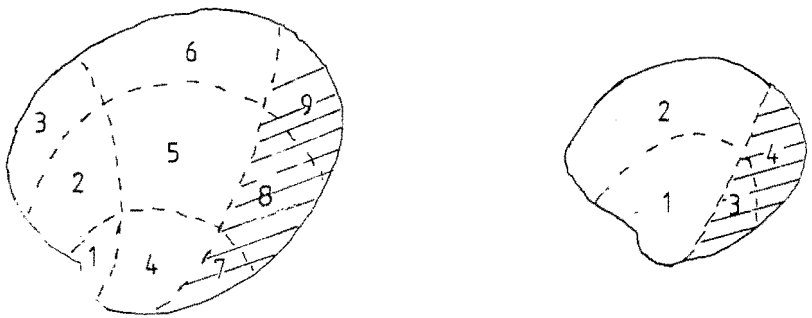
(ii) The lead concentration in the area of shell that is etched by sand movement is considerably lower than for the portion of the shell which remains below the sand/water interface (see Figure 3.6).

(iii) When the highest concentration on the shell surface is plotted as a function of shell length, which is related to shell age, the graph obtained is best described as exponential with shell length (see Figure 3.7), that is, the older the shellfish the greater the surface lead concentration. These observations support the suggestion that the lead on the shell surface has primarily accumulated by absorption of lead ions from the estuarine environment. The lead concentrations are highest in old shell and much lower in the area where the surface is being continuously

Figure 3.4

Diagram Showing the Division of the Shell Exterior Surface into Zones

For the Investigation of Element Variation over the Surface.



≡ Area Etched by Sand

Table 3.4

Distribution of Lead Within Zones on the Exterior Surface of Shells.

Large Shells

	Shell					
Zone	1	2	3	4	5	6
1	38±1	44±4	114±26	123±15	29±5	59±5
2	20±3	22±3	21±3	47±5	6±2	36±2
3	6.4±0.5	9±1	16±1	25±2	4.5±0.7	6.7±0.7
4	34±3	23±2	54±6	41±1	9.4±1.0	39±10
5	16±1	18±2	25±2	20±4	15±3	24±4
6	8.1±0.2	9.5±0.9	4.0±0.8	2.2±0.2	5.6±0.5	8.3±1.5
7	6.1±1.0	5.7±0.6	2.6±0.4	6.6±0.6	4.0±0.1	8±2
8	3.1±0.4	6.3±1.2	0.7±0.2	1.7±0.2	9.0±0.9	7.7±0.7
9		2.5±0.3	0.4±0.1	1.1±0.1	6.5±0.7	3.9±0.9
Shell Length	43mm	41mm	45mm	46mm	38mm	37mm

Table 3.4 cont.

Small Shells

	Shell			
Zone	1	2	3	4
1	15±2	13.3±0.9	6.3±0.2	8.1±0.7
2	3.7±0.5	4.8±0.3		
3	3.5±0.2	2.6±0.2	6.2±0.3	5.2±0.6
4	2.0±0.2	11.7±0.2		
Shell Length	28mm	29mm	25mm	26mm

Notes: (1) Zones are as in Figure 3.4.

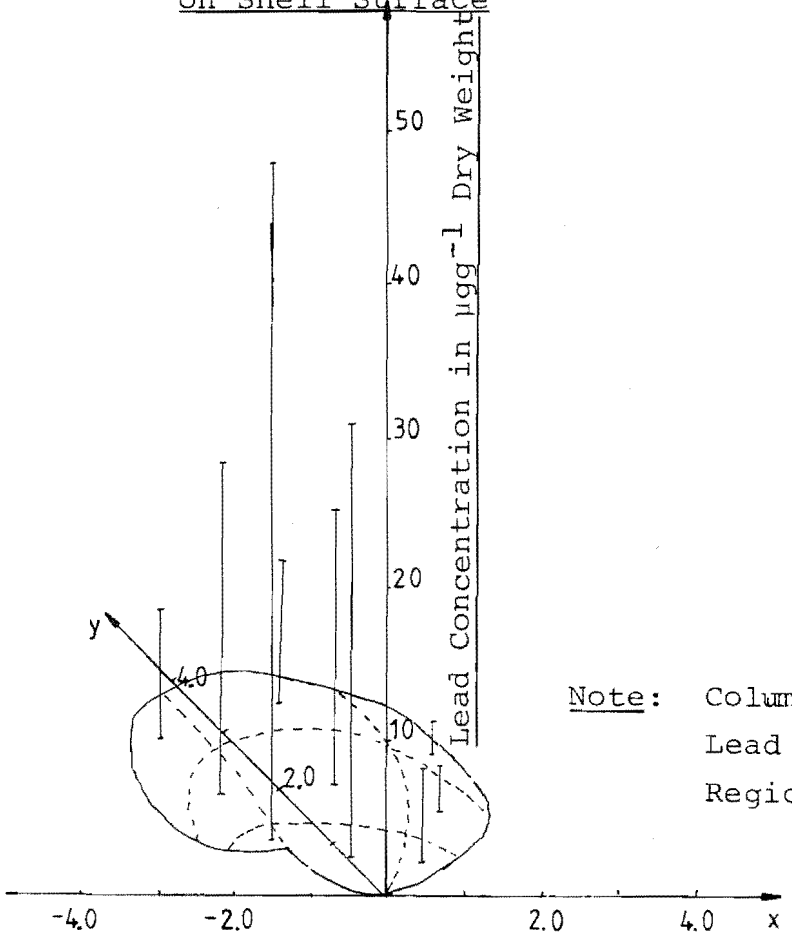
(2) Lead concentration is in $\mu gPbg^{-1}$ dry weight.

(3) Blanks in table due to values not measured.

(4) Values are mean±error.

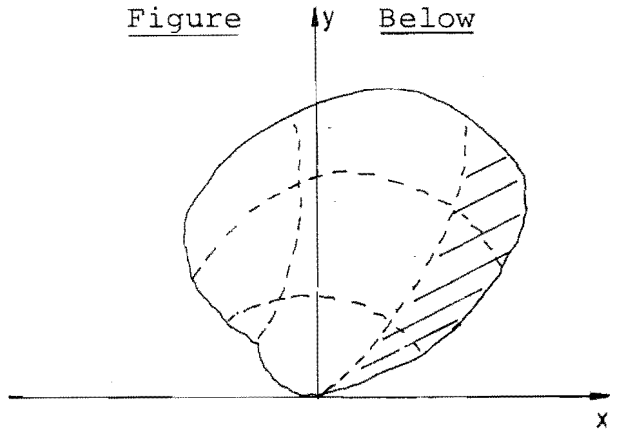
Figure 3.5

Lead Concentration as a Function of Position on Shell Surface



Note: Column Length Represents Lead Concentration For Each Region

Diagram Showing Sampling Zones for Figure Below



Area Etched by Sand Movement

Figure 3.6

Diagram Showing Position of Chione (Austrovenus) stutchburyi in the Sediment

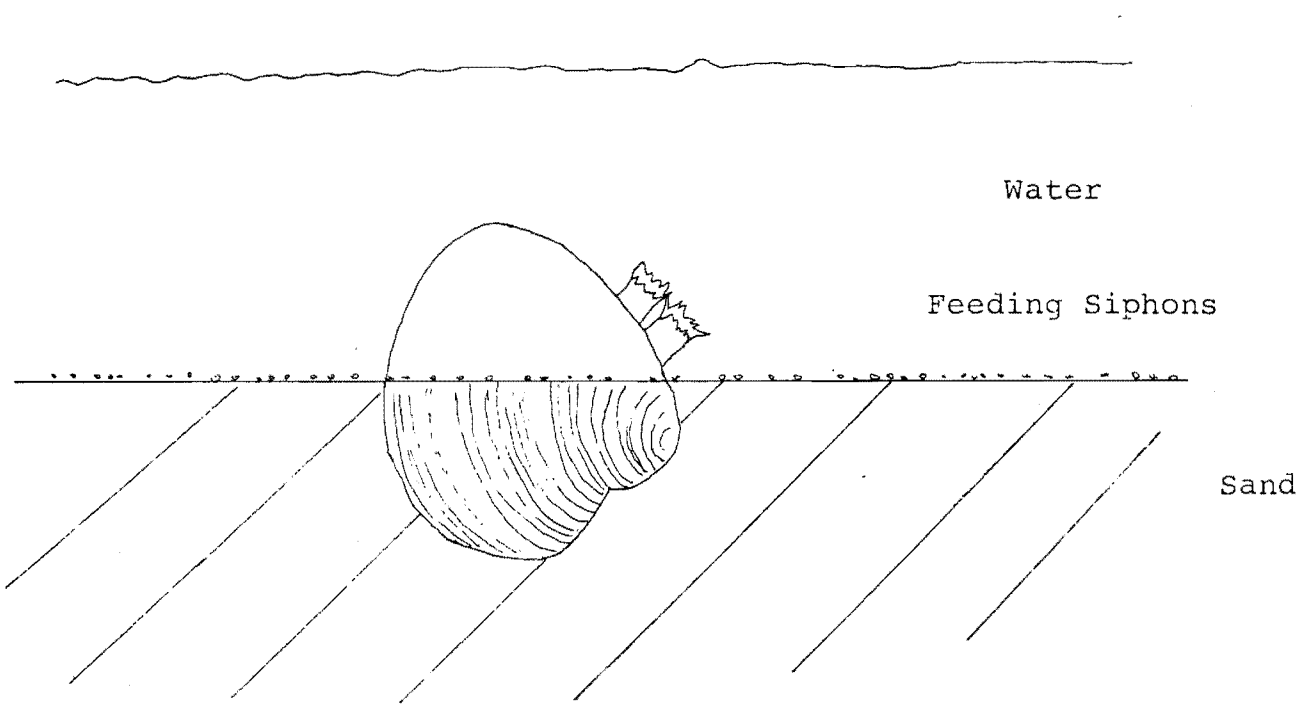
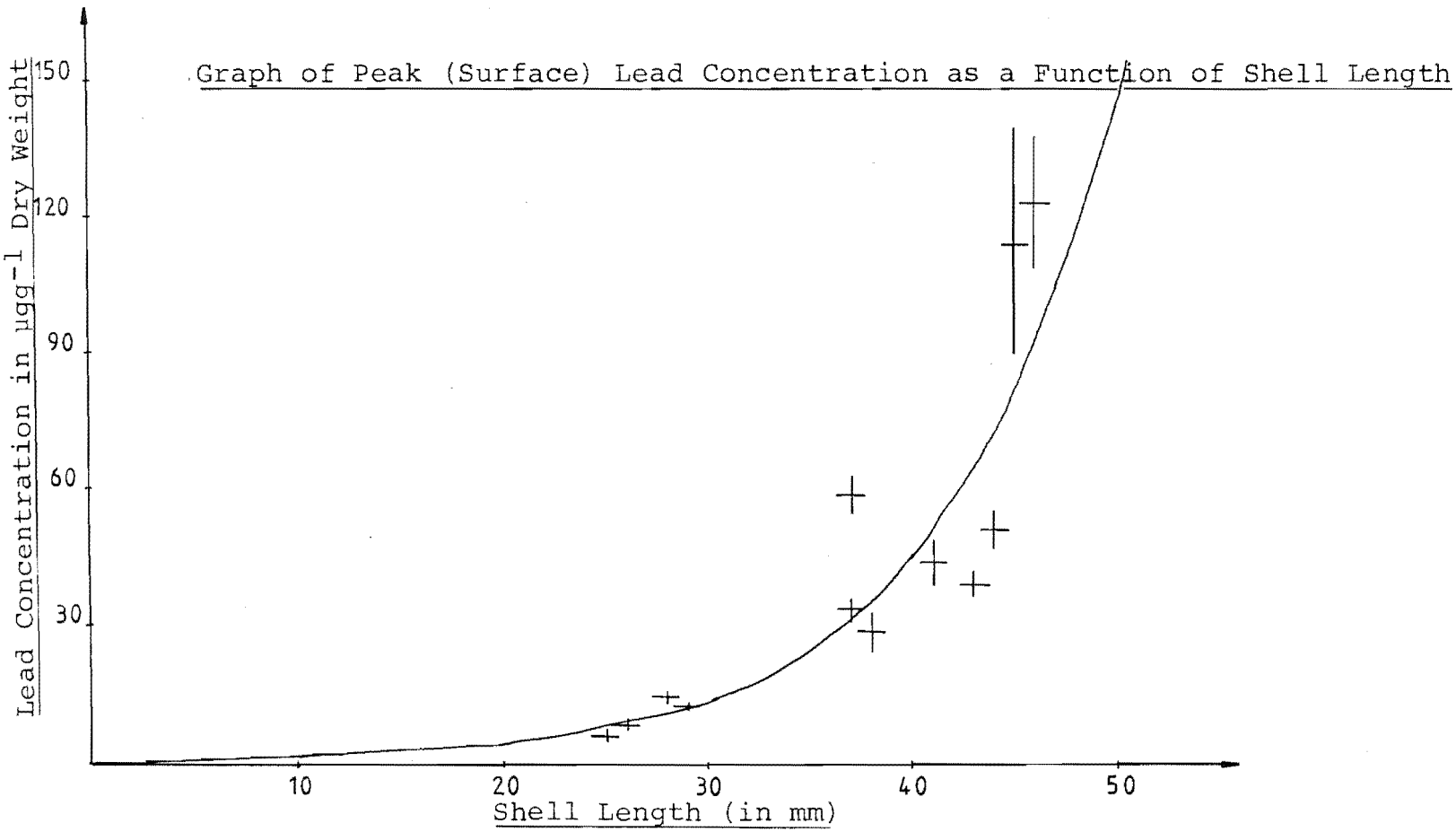


Figure 3.7


Graph of Peak (Surface) Lead Concentration as a Function of Shell Length
Lead Concentration in µgg-1 Dry Weight
150
120
90
60
30
10
20
30
40
50
Shell Length (in mm)

etched. To see if this occurred for other elements, the levels of copper, chromium and zinc were measured in a similar way to the lead concentrations and the results are given in Table 3.5.

For zinc, there appears to be no systematic pattern of local concentration, suggesting surface absorption makes only a minor contribution to the total shell burden of zinc. Also the data for copper has no clear pattern, but it would appear the surface copper concentrations are higher in the etched region of the shell, otherwise there is little evidence for an important contribution from absorption to the overall burden of copper in the shell. For chromium, the data is suggestive of some surface absorption taking place, as older shell has a higher surface chromium concentration than the newly formed shell near the posterior edge (see Figure 3.8). However, the effect of etching is not as great as with lead which would suggest that incorporation during growth is a more important pathway for chromium deposition in the shell.

A possible explanation for the difference in the level of importance for surface absorption for lead compared with copper, chromium and zinc, may be in the structural features of calcium carbonate. Calcium carbonate can exist either as rhombohedral calcite or orthorhombic aragonite. The infrared spectra of a Chione (Austrovenus) stutchburyi indicated that the $CaCO_3$ structure was entirely aragonite. (See Figure 3.9). For metal ion carbonates MCO_3 the calcite structural form occurs for cations whose ionic radii are less than 98pm while the aragonite structure occurs for

Table 3.5

Variation of Trace Element Concentration over Shell Surface (zones as in Figure 3.3 for Large Shells).

	Chromium			
	Shell			
Zone	1	2	3	4
1	8.2±1.3	8.0±1.2	14.6±2.1	12.9±1.5
2	3.2±0.3	9.4±0.5	7.2±0.4	7.1±0.8
3	4.3±0.2	3.8±0.2	6.2±0.3	4.0±0.7
4	5.5±0.3	7.6±1.1	8.3±1.0	7.4±0.7
5	7.5±1.0	4.8±0.2	10.7±1.7	8.5±0.6
6	5.6±1.0	5.4±0.6	4.1±0.1	2.3±0.1
7	13.9±2.8	5.4±1.4	8.8±0.6	4.8±0.3
8	9.5±2.0	7.2±1.1	3.5±0.3	4.4±0.4
9	6.8±0.6	4.0±0.6	2.5±0.1	2.9±0.3
Shell Length (mm)	38	37	45	46

Table 3.5 cont.

	Copper			
	Shell			
Zone	1	2	3	4
1	21±5	8.9±1.5	5.7±0.4	15.6±1.6
2	5.0±0.3	10.3±1.5	4.0±0.4	7.1±0.4
3	4.4±0.2	5.9±0.5	9.2±0.9	4.7±0.1
4	11.3±1.1	8.9±0.7	4.1±1.2	7.8±0.9
5	7.1±0.7	5.1±0.7	6.2±1.7	6.1±0.4
6	7.5±0.9	6.5±0.6	9.7±1.5	8.0±0.3
7	21±3	14.1±1.0	15.6±2.9	19.7±5.0
8	9.1±1.1	10.2±0.6	16.9±1.7	6.9±0.5
9	47±4	21.5±2.0	14.6±1.3	9.2±0.5
Shell Length (mm)	45	46	38	37

Table 3.5 cont.

Zinc

Shell

Zone	1	2	3	4
1	43±3	16±3	37±4	3.5±3
2	10±1	47±2	18±1	30±2
3	11±1	16±0.5	25±0.5	25±1
4	27±2	9±1	27±1	24±1
5	13±2	91±1	26±1	19±1
6	19±2	10±1	25±1	41±1
7	42±8	12±3	44±3	28±1
8	91±7	14±1	24±2	41±5
9	35±3	7±1	39±1	33±1
Shell Length (mm)	38	37	45	46

Notes: (1) Chromium concentration in $\mu gCr g^{-1}$ dry weight. (2) Copper concentration in $\mu gCu g^{-1}$ dry weight.
(3) Zinc concentration in $\mu gZn g^{-1}$ dry weight. (4) Values are mean±error.

Figure 3.8

Chromium Concentration as a Function of Position on Shell Surface.

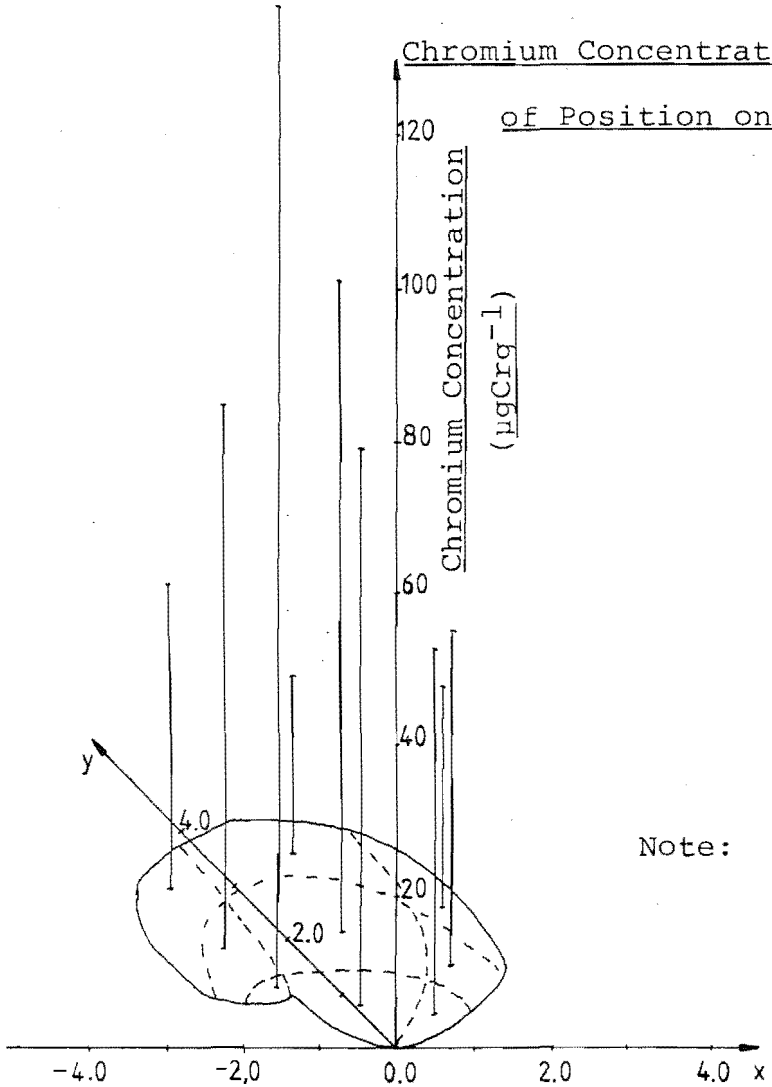
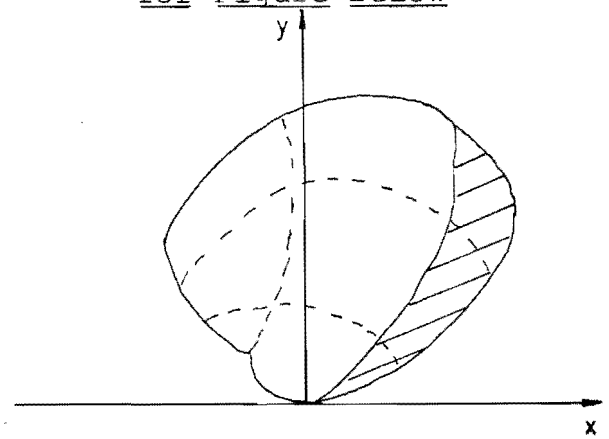


Diagram Showing Sampling Zones for Figure Below

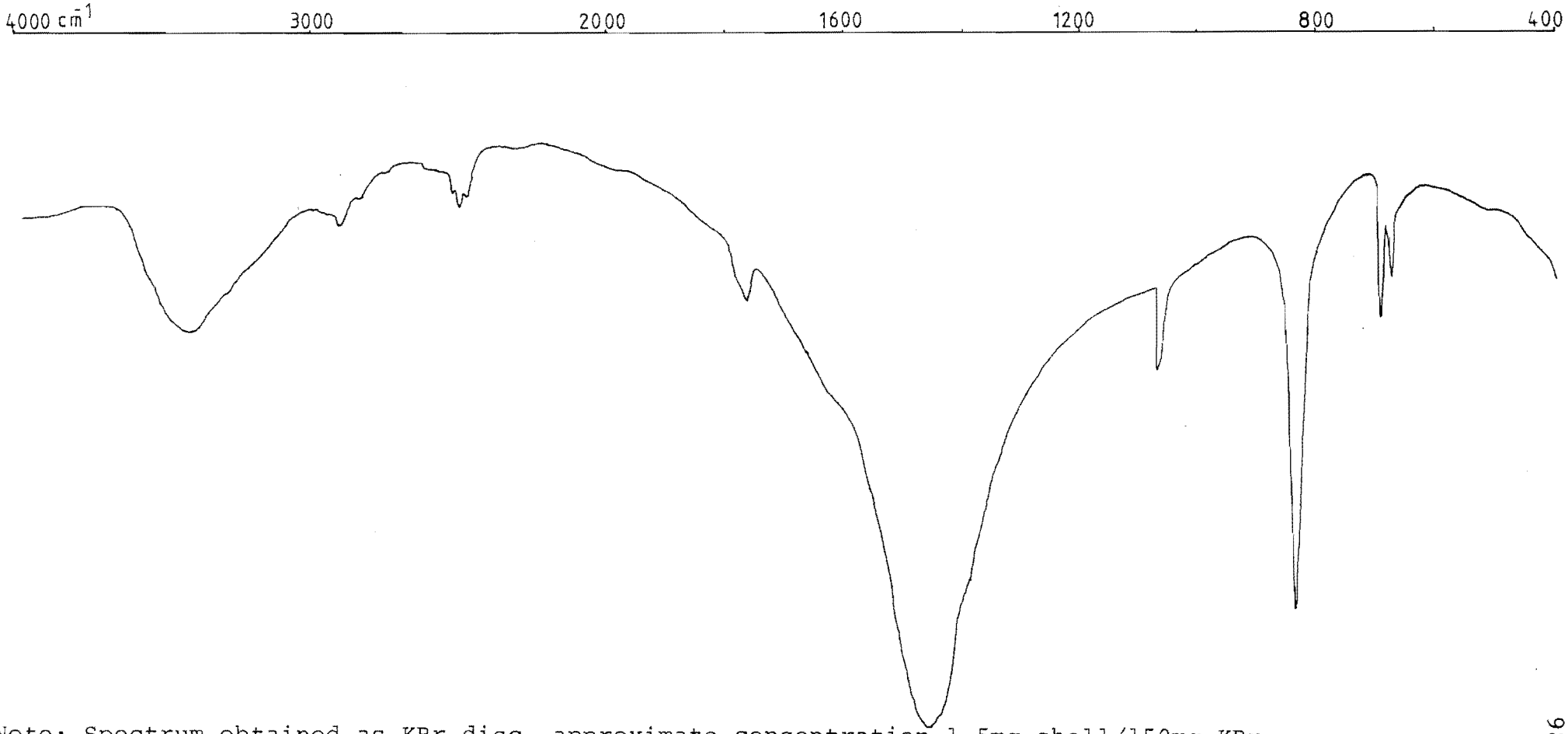


Area Etched by Sand Movement

Note: Column Length Represents Chromium Concentration for Each Region

Figure 3.9

Infrared Spectrum of Shell Powder from the Shellfish Chione (Austrovenus) stutchburyi.



Note: Spectrum obtained as KBr disc, approximate concentration 1.5mg shell/150mg KBr.

cations whose radii are greater than 98pm. The ionic radius of Pb^{2+} is 120pm whereas the values for Cu^{2+} , Cr^{3+} and Zn^{2+} are all less than 80pm. This would suggest that the lead ion would be more easily incorporated into the aragonite structure than copper, chromium or zinc. This might explain why absorption is more important for lead than for the other metals, especially if absorption is followed by isomorphous ionic replacement in the shell structure.

3.4.1 Conclusion

For lead in the shells of Chione (Austrovenus) stutchburyi both incorporation in the shell matrix with deposition of the shell and ionic isomorphic replacement following surface absorption have been demonstrated. The analysis of a cross sectional portion of shell has shown that lead is incorporated during the growth of the shell and the lead concentration reflects the available lead to the shellfish at the time of deposition. The lead concentration in newly laid down shell was found to be lower than the lead concentration in the whole soft tissue on a dry weight basis. Hence the variation in lead concentration in going from the newly laid down shell to the outer shell presents a record of lead availability to the shellfish. However, it must be noted that shell growth only occurs over the summer months, and therefore the record of lead availability from shell measurements only gives part of the total record.

Isomorphous replacement after surface absorption has

been suggested as a second mode of incorporation. It has been demonstrated that the lead concentration on the shell surface is dependent on the age of the shell, new shell having lower lead concentrations than older shell. Hence the outer shell gives an integrated index of lead exposure over the time of the shell's life. Surface absorption was also shown to be of some importance for chromium, but not as great as for lead. The evidence for copper and zinc suggests that surface absorption does not play a significant part in the total burden of these elements in the shell.

References.

1. Sturesson, U. Lead enrichment in shells of Mytilus edulis. Ambio, 5, 253-256, (1976).

2. Sturesson, U. Cadmium enrichment in shells of Mytilus edulis. Ambio, 7, 122-125, (1978).

3. Goldberg, E.D; Bowen, V.T; Farrington, J.W; Harvey, G; Martin, J.H; Parker, P.L; Risebrough, R.W; Robertson W; Schneider, E; Gamble, E. The mussel watch. Environ. Conserv., 5, 101-125, (1978).

4. Chow, T.J; Snyder, H.G; Snyder, C.B. Mussels (Mytilus sp.) as an indicator of lead pollution. Sci. Total Environ., 6, 55-63, (1976).

5. Mackie, A.C. Studies on the measurement of environmental lead. PhD. Thesis, University of Birmingham, U.K., (1975-1976).

6. Fergusson, J.E; Jansen, M.L; Sheat, A.W. Lead in deciduous teeth in relation to environmental lead. Environ. Technol. Lett., 1, 376-383, (1980).

7. Chow, T.J; Snyder, C.B; Snyder, H.G; Earl, J.L. Lead content of some marine organisms. J. Environ. Sci. Health - Environ. Sci. Eng., A11, 33-44, (1976).

8. Clark, J.H; Clark, A.N; Wilson, D.J; Friauf, J.J. Lead levels in fresh water mollusk shells. J. Environ. Sci. Health - Environ. Sci. Eng., A11, 65-78, (1976).

Chapter 4

The Distribution of Heavy Metals within the Avon-Heathcote Estuary System, and Some Investigation of the Chemistry of Lead within this System

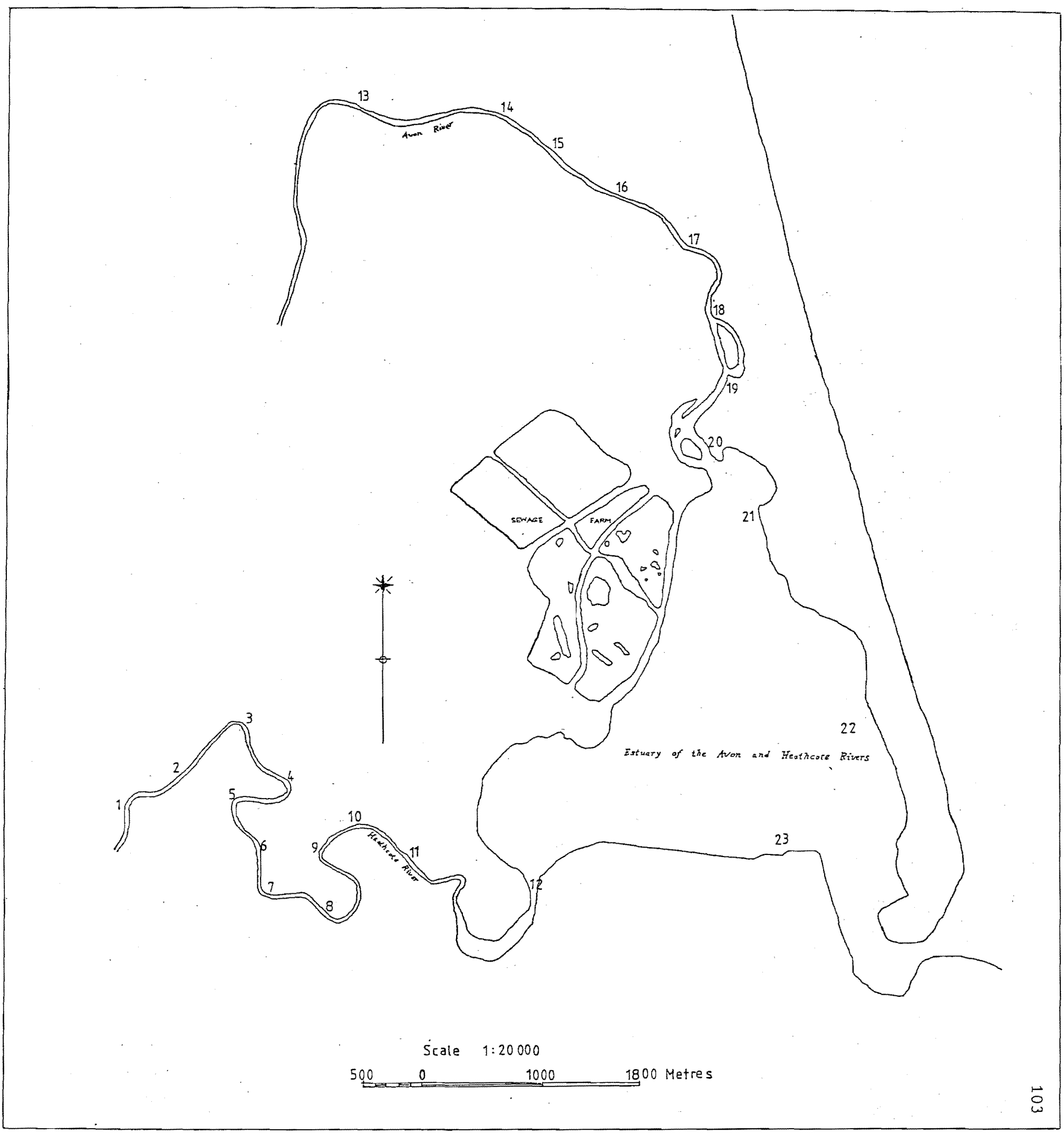
4.1.1 Introduction

The use of shellfish as a monitor of lead pollution within the Avon-Heathcote Estuary was discussed in Chapter 2. It was suggested that a reason for the fall in lead concentrations within shellfish with time could be attributed to a reduced lead availability to the organisms in the estuary. Since the use of automotive lead has not decreased, it would appear that an industrial source was involved. These considerations prompted an investigation of lead concentrations in the sediments of the Avon and Heathcote Rivers, as well as some areas within the Avon-Heathcote Estuary.

An additional aim of this work was to investigate an hypothesis of Macpherson (1), that heavy metals are not accumulating within the Avon-Heathcote Estuary. This is because the volume of water in the estuary was shown to be increasing, therefore the estuary was self flushing. However, Macpherson suggested that some accumulation of material may be occurring near Pleasant Point (Figure 4.1, Site 21) and sediment profile analysis was undertaken to see if this was so. Also, as a check to make sure that the levels of lead in the shellfish (Chapter 2) were not influenced by nearby local sources of lead, samples of sediment, road dust and some vegetation were collected

Figure 4.1

Map of the Sediment Sampling Sites within the Avon-Heathcote System.



and analysed from near the shellfish sampling site.

Tan (2) had measured lead and cadmium concentrations in the surface sediments of the Heathcote River and the Avon-Heathcote Estuary, but not in the Avon River. However, it was considered important to look at some sediment profiles especially from highly polluted areas of the Heathcote River. This would also allow for an investigation into concentration difference with depth to see if there was any temporal variation. It was also decided to undertake analysis for a number of other trace elements in the profiles viz. antimony, cadmium, chromium, copper, iron, manganese and zinc.

The reason for the particular choice of these elements is the nature of some industries along the river. Lead and antimony are used in the manufacture of lead-acid batteries and a battery factory is located on the banks of the Heathcote River. Zinc is used in the manufacture of rubber products (and by association cadmium, due to its chemical similarity with zinc) in a factory adjacent to the battery factory. Chromium was investigated because of the presence of several tanneries on the banks of the Heathcote River. Iron and manganese were determined as many trace elements are associated with the hydrous oxide forms of these two metals.

As high levels of lead were found in the sediments, it was thought worthwhile to investigate how lead was "fixed" in the sediment. This study involved two areas of investigation. Firstly, river sediment of low lead concentration was used in a sorption study to investigate the mechanism of lead sorption onto sediment, especially

the clay fraction of the sediment. In the second study, different fractions of the sediments were studied in order to determine the nature of the lead chemical species in the sediment.

At the time that the sediment investigations were being carried out, much interest was aroused in different laboratories by the possibility of bio-methylation of inorganic lead to tetramethyl lead. While bio-methylation had been suggested to occur in laboratory experiments (3-6), of considerable interest was the suggestion by Harrison and Laxen (7) that natural environmental methylation of lead was occurring in the intertidal mudflat area near air sampling sites they had chosen. However, from more recent evidence it has been suggested that bio-methylation does not occur, and the previous results were due to chemical processes and not biological (8, 9) and that bio-alkylation of Pb(II) does not occur.

To investigate the presence of organic Pb(IV) species (especially trimethyl and tetramethyl lead) cockles (Chione (Austrovenus) stutchburyi) were collected and a method developed in an attempt to measure the presence of these compounds in the Avon-Heathcote Estuary. However, this experiment could not give any information on whether bio-methylation of lead occurred, but would only indicate the level of organolead compounds present.

4.2 Analytical Methods and Sample Preparation

4.2.1 Methods for Dissolution of Sediment Samples.

After sediment samples had been collected, they were

oven dried at 85°C for 48 hours, as this was found to be sufficient time for drying to constant weight. Samples were crushed in an agate pestle and mortar until they would pass through a 500μm sieve. They were then placed in pre-acid washed glass jars for storage until analysis.

If the only analysis required on the sample was for lead, then a weighed sample was digested in 2M nitric acid ("AnalaR" grade), for approximately 30 minutes on a hot plate with the solution mixture as near boiling as possible but not so hot that "bumping" occurred. The solutions were then filtered through Whatman 40 filter papers.

Ten samples were reboiled in 2M nitric acid to see if the first extraction had removed all the lead in the sediment. In no case did the second extraction contain more than 5% of the total lead extracted. Fergusson and Simmonds (10) using the same extraction technique found an average of 98.9% extraction for the first extraction. Fergusson and Simmonds (10) also used a $HF/HC1O_4$ extraction, the same as was used for multielement analysis and found that the extraction of lead by boiling in nitric acid was 95-100% of that obtained by the $HF/HC1O_4$ extraction.

If the sediment was to be analysed for more elements than lead it was dissolved by digestion with a hydrofluoric/perchloric acid mixture. Before digestion the samples were ashed at 400°C for 16 hours to remove all combustible organic material; this also allowed for a calculation of the percentage of organic material to be made. Approximately 0.25 g of the ashed sediment was placed into a platinum crucible and mixed with 5ml of concentrated hydrofluoric acid and 1mL of concentrated perchloric acid. The crucibles

were then placed in a sand bath (at approximately 200^{o}C) and the solutions heated to dryness and until fuming had ceased (approximately three hours). The residue was then dissolved in 5mL of 2M nitric acid by heating for 30 minutes, and the resulting solution quantitatively washed into a volumetric flask.

4.2.2 Densiometric and Magnetic Separation of Sediment Samples.

Densiometric separation was carried out on some sediment samples with the aim of isolating a fraction with a high lead concentration (generally the more dense fraction (11)). This separation was performed by "floating" 10 g of sediment in 50mL of tetrabromoethane (TBE) in a separating funnel. The material of density greater than that of TBE (2.95gcm^{-3}) was collected and washed with ethanol until no TBE remained. The sample was then dried at 85^{o}C for 16 hours. In order to concentrate the lead fraction further, the sediment with density greater than 2.95gcm^{-3}, was fractionated by removing magnetic material with the aid of a permanent magnet.

4.2.3 Separation of Sediment Samples into Sand, Silt and Clay Fractions.

To investigate the partition of trace elements between sand, silt and clay fractions, sediment samples (which had been carefully crushed to break up large lumps) were separated into these fractions. For the purpose of this

investigation, the following size limits were used to define the three fractions: (i) sand was defined as particles > 20μm (ii) silt was defined as particles 2 - 20μm and (iii) clay was defined as being made up of particles< 2μm.

To carry out this separation, the average density of the sediment had to be found. This was done by volume displacement using water as the medium. The average density found by this method was approximately $2.22 gcm^{-3}$. To separate the fraction by centrifuging use was made of the relationship between the speed of the centrifuge, the density of the sediment and the physical dimensions of the centrifuge (12).

$$\frac{t}{\frac{R}{\log(S)}} = \frac{K_c}{N^2 D^2}$$

This relationship may be obtained from Stokes Law:

$$t = \frac{K_c \log(\frac{R}{S})}{N^2 D^2}$$

where N is the speed in rpm of the centrifuge, D is the average density of the sediment, R is the distance from the centre of the centrifuge to the top of the suspension in the sample tube, S is the distance from the bottom of the suspension to the centre of the centrifuge, t is the time for settlement of the particles of the size required and K_c is a constant. From tables (12), the following values were used for this separation, for R=20cm, and S=10cm for the centrifuge being used, and for 10cm height of

suspension. For particles greater than 2μm, t=180 sec and N=1500 rpm. For particles greater than 20μm a fall of 10 cm took six minutes under gravity.

To accomplish the separation of the sediment into sand, silt and clay fractions, approximately 10g of sediment were placed in a plastic shaking tube and water added to a height 10 cm above the sediment. The tube was then sealed with a plastic coated bung and vigorously shaken. To aid in the dispersement of the clay particles, a 25mL aliquot of 4% sodium hexaphosphate was used. After shaking, the suspension was spun at 1500 rpm (set by a General Radio Xenon Strobe) for 180 seconds and the supernatent fluid siphoned off. The process was continued until the supernatent fluid was clear. The siphoned suspension contained the clay fraction.

The silt and sand were separated by shaking the remaining sediment with water and after the suspension has been allowed to settle under gravity fall for six minutes, the supernatent fluid was siphoned off. This was repeated until the supernatent fluid was clear. The fraction left in the tube was classified as the sand fraction. The silt was obtained from the siphoned suspension by centrifuging.

To separate the clay fraction from its suspension, a saturated solution of salt (NaCl) was added, causing the clay particles to flocculate and settle to the bottom of the vessel. The clear supernatent fluid was decanted off and the clay partciles washed by decantation until they began to disperse again. The material was then placed in a centrifuge tube, a small amount of saturated salt

solution (just enough to bring about flocculation) and 4% alcohol were added and the clay suspension centrifuged at high speed. The clear supernatent fluid was decanted and the clay fraction was freeze-dried.

The silt and sand fractions were washed with distilled water and then spun down in a high speed centrifuge with a solution containing 4% alcohol. They were then oven dried at 85°C for 16 hours.

The recovery of material at the end of this process was between 92% and 97%, giving an average loss of material of approximately 5%. The average volume of liquid used (in total) in the separation of 10g of material was approximately 2L. The loss of some material was due to the presence of organic matter which floated on top of the liquids.

4.2.4 Methods for Identification of Lead Compounds in Sediments.

Three methods were employed to determine the type and amount of various lead compounds in the sediment samples. The first of these was X-ray powder diffraction. For this method, a finely powdered sample of the heavy material obtained by magnetic and densiometric separation from the sediment sample was placed in a fine glass capillary tube and mounted in an X-ray (Debye-Scherrer radius=5.73cm) camera. The recording medium used was a photographic emulsion with the light source being copper radiation from the K_α line (λ =0.15404nm). The 2θ values were measured and the d-values were calculated and these

compared with data from the J.C.P.D.S. powder diffraction file (13). The films were also compared directly with X-ray powder diffraction photos taken of known lead compounds. While this method gave an indication of which compounds were present, it did not give a good indication of the relative amounts of each compound. A photographic method was used rather than X-ray powder diffractograms because of, in some cases, the small amount of material available.

The second method used was quantitative IR using a Pye Unican SP3-300 Infrared Spectrophotometer. While it was possible to get a good calibration for compounds which had an absorbance in the 2000-200cm^{-1} region, problems occurred for the actual sediment samples. Up to 50% of the sample was various silicates and some organic matter and while this made no difference for X-ray powder diffraction, the presence of these two materials made correction for background curvature so difficult, that meaningful results could not be obtained by infrared spectroscopy. Also, only two of the lead compounds indentified by powder X-ray diffraction were observable in the working range of the instrument.

The third method used for looking at lead compounds in sediments was that of differential thermal analysis (DTA). In DTA, the heat of reaction, when a compound present undergoes structural or chemical changes, is measured against a stable compound on which heating has no effect. There are two reaction types, namely exothermic (causing the sample to be hotter than the reference) or endothermic (causing the sample to heat more slowly than the reference). For this work, the reference and dilutant compound was

kaolinite which had previously been heated to 1200°C. This method allowed for quantitative measurement of the amount of each lead compound present in the sediment. The analyses had to be carried out in still air and therefore some allowance had to be made for the oxidation of organic matter present.

The analyses were run on a DTA instrument at the Soil Science Department, Lincoln College. The temperature range was from room temperature to 1000°C. Normally 40mg of each sample was used, (in one case only 20mg was available). The sensitivity of the instrument was set at 1°C per inch (2.54cm) and the output plotted on an X-Y chart recorder from which all measurements were later calculated.

4.2.5 Determination of Clay Types by Powder X-ray Diffraction.

In order to determine the mineral make up of the clay fraction, X-ray powder diffraction was employed. Firstly, the clay material was ground in an agate pestle and mortar using ethanol as the grinding medium. A small quantity of the resulting slurry was then placed on a glass microscope slide and then left to dry overnight at room temperature. The slides were then mounted in a powder X-ray diffractometer in the Geology Department (University of Canterbury), and the diffractograms run. The samples, while still on their glass slides, were then glycolated with ethylene glycol. This produces swelling in some types of clays and allows for the differentiation of montmorillanite

(which swells) from chlorite (which does not). The diffractograms were again run and the diffraction pattern recorded. The final step in the clay analysis, was the heating of the samples on their glass slides to 500°C for one hour. This was done to check if the peak due to kaolinite was due to just kaolinite and not to a secondary chlorite peak. On heating, the kaolinite is transformed into poorly ordered metakaolinite which does not produce a diffractogram (14). The samples were therefore run for a third time and the peaks on the diffractograms assigned to their respective compounds using data from the J.C.P.D.S. powder diffraction files.

4.2.6 Analysis for the Presence of Organolead Compounds in Shellfish.

The shellfish used was the New Zealand cockle (Chione (Austrovenus) stutchburyi), which is the same species as that used for the monitoring of lead burdens in the Avon-Heathcote Estuary (see Chapter 2, Section 2.1.6 (a)). The site chosen was the same as for the temporal study done with shellfish in the Avon-Heathcote Estuary (see Section 2.3.5).

After the shellfish were collected, they were allowed to stand for 24 hours to allow for defecation to occur so that any solid material held in the animal gut would be lost by natural processes. They were then removed from their shells and the wet weight of each animal recorded. To liberate the available lead compounds and complexes from the biological material of the shellfish, the shellfish

was placed in a "Virtis-45" high speed blender with the aim of homogenising the animal material with redistilled water. However, this was not found to be satisfactory as analysis of centrifuged biological material showed that approximately 90% of the lead was still bound to the biological material.

To improve the dispersion of lead complexes and compounds from the biological material, after the shellfish material had been "homogenised" in the blender, it was then incubated with the enzyme pancreatin (which breaks up proteins) for 24 hours at 37^{o}C. After this treatment, centrifuged biological material contained less than 30% of the total lead.

Solvent extraction on the resulting material was used as the method for extracting tri-alkyl and tetra-alkyl lead compounds. Tetra-alkyl lead compounds were extracted with benzene, while tri-alkyl lead compounds were extracted with ethyl acetate. For the extraction 5mL of the benzene was added to the incubated mixture and sealed with a plastic bung. The tube was then shaken for 15 minutes and then the resulting emulsion was spun at 2500 rpm for 10 minutes and the benzene layer removed, a second 5mL of benzene was added, shaken and spun as for the first addition of benzene. To extract tri-alkyl lead, the suspension had to be salted with 10mL of saturated salt (NaCl) solution to increase the extracting ability of the solvent ethyl acetate. For the tri-alkyl lead extraction, two 5mL portions of ethyl acetate were shaken for 15 minutes and spun at 2500 rpm for 10 minutes and the solvent layer collected. This solvent extraction technique is based on the extraction

method for tetra-alkyl lead and tri-alkyl lead compounds developed by Hayakawa (15).

To ensure that the extracting solvents were free from lead compounds, the "AnalaR" grade reagents had been previously shaken with a 2% EDTA solution, and then twice distilled in an all glass still. Double distilled water was used throughout and this produced blanks with absorptions of the same order as the instrument noise levels. Analysis was carried out by graphite furnace atomisation atomic absorption spectrophotometry (GFA-AAS) using carbon cups, as these were better at containing the organic solvents where solvent "creep" is a problem.

In order to check on the efficiency of the extraction method, an addition of 50ng of lead as $(C_2H_5)3PbCl$ was added to the system, and after incubation, extraction with benzene and extraction with ethyl acetate, recovery was found to be approximately 97% with a range of 94-100%. Extraction occurred in the ethyl acetate fraction, as expected.

4.2.7 Instrument Settings for Atomic Absorption Spectrophotometry.

For the determination of trace elements in sediments, atomic absorption spectrophotometry was used.

For chromium, iron and manganese, a nitrous oxide and acetylene flame was used, as these elements are not adequately atomised in the air and acetylene flame. Copper, lead and zinc were determined using an air and acetylene flame. Because of the relatively poor sensitivity of

antimony for atomic absorption spectrophotometry, graphite furnace atomisation was employed, while because of the low concentration of cadmium in the sediments, GFA-AAS was also used for its determination.

All reagents were of "AnalaR" grade or better, in order to keep reagent contributions to the blanks as low as possible. Also for the analyses using flame atomisation, a deuterium lamp was employed to allow for automatic correction of background, due to scattering in the flame from refractory species not being analysed for at the time.

To check to see if there was interference during analysis or loss during digestion, trace elements were added to the ashed samples prior to digestion. This allowed a check to be made by the method of standard additions. This showed that interference did not occur under the analytic conditions chosen and that there was no loss of elements during the digestion processes (see Table 4.1).

Table 4.1

Percentage Recovery of Elements by Standard Additions

Element	Mean	Standard Deviation	Range
Antimony	96	6	86-100
Cadmium	98	2	97-101
Chromium	114	7	109-122
Copper	88	18	72-118
Iron	93	8	80-105
Manganese	90	7	83-98
Lead	101	4	96-107
Zinc	95	10	80-110

All analyses were carried out on a Varian AA-1475 atomic absorption spectrophotometer. The instrument settings are given in Table 4.2. For graphite furnace atomisation a Varian CRA-63 carbon rod atomiser was used. Settings for this instrument are also included in Table 4.2.

4.2.8 Sorption of Lead onto Sediments.

In order to investigate the sorption properties of the sediments with respect to lead, sorption experiments were carried out on a river sediment sample which had a low lead concentration. Because lead salts precipitate at the pH of natural river water, the system was buffered by 0.02M sodium acetate/acetic acid buffer pH=4.74. A solution of a known quantity of added lead salts and buffer was added to flasks containing identical quantities of sediment and left in a thermostat bath at $25 \pm 0.5^{\circ}C$ for 24 hours. Analysis of the lead remaining in solution was carried out by air/acetylene atomised AAS.

4.2.9 Sample Collection and Initial Handling.

Sediment was collected from sites within the Avon-Heathcote Estuary and the lower reaches of the Avon and Heathcote Rivers. A map showing the various sampling sites is given in Figure 4.1.

Surface sediments were obtained by scooping up the top 1-2 mm of sediment with a small plastic scoop and placing the sediment in numbered plastic sample bags. The profiles were obtained by sinking a polythene tube with

Table 4.2

Instrument Settings for Analysis of Sediment Material.

Varian AA-1475	Cd	Cr	Cu	Fe	Mn	Pb	Sb	Zn
Wavelength (nm)	228.8	357.9	324.8	248.3	279.5	217.0 283.0	217.6	213.9
Slit Width	0.5	0.2	0.2	0.2	0.2	1.0 0.5	0.2	0.2
Lamp Current (mA)	3	5	3	5	5	5	10	5
Background Correction	No	Yes	Yes	Yes	Yes	Yes[1]	No	Yes
Mode of Atomisation	CRA	C_2H_2/N_2O	C_2H_2/Air	C_2H_2/N_2O	C_2H_2/N_2O	C_2H_2/Air	CCA	C_2H_2/Air
Standard Range[2]	10-25	0.25-40	0.25-50	7-100	0.7-10	0.5-100	1-10	0.2-1.6

Varian CRA-63	Cd	Cr	Cu	Fe	Mn	Pb	Sb	Zn
Dry (Time)	4.5 (10)					4.5 (10)	4.5 (10)	
Ash (Time)	3.0 (5)					5.0 (5)	3.0 (5)	
Atomise	Ramp					Ramp	Ramp	

Table 4.2 cont.

Varian CRA-63	Cd	Cr	Cu	Fe	Mn	Pb	Sb	Zn
Ramp Rate	3.0					3.0	3.0	
Votage Cut Off	7.0					7.0	7.0	

Notes: (1) Background Correction only used during flame analyses.

(2) Standards in μgmL^{-1} except Cd where in $ngmL^{-1}$.

(3) CRA means carbon rod.

(4) CCA means carbon cup.

(5) C_2H_2means acetylene, N_2O means nitrous oxide.

approximately 4 cm internal diameter and 60 cm length, into the sediment and removing the core from the sediment. Sampling sites were reached by foot at periods of low tide so the covering water was kept to a minimum above the sediment.

On returning to the laboratory, the surface sediment samples were placed on acid washed, large watch glasses (10 cm in diameter), and allowed to air dry before being dried at 85°C for 16 hours to obtain a dry sample. For the sediment cores, on returning to the laboratory, these were extruded onto polyethylene sheet plastic and allowed to air dry. The sediment cores were then divided into sections before being oven dried at 85°C for 16 hours. All sediment samples were stored in glass jars with screw top lids to ensure that atmospheric fallout did not affect the levels of trace elements within the samples.

4.3. Results and Discussion

4.3.1 Survey of Lead Concentration in Surface Sediments of the Lower Reaches of the Avon and Heathcote Rivers and the Avon-Heathcote Estuary.

Lead concentrations in surface sediments from the Avon-Heathcote River system are given in Table 4.3 and depicted graphically in Figures 4.2 and 4.3. From these results, several trends are apparent. The first is that lead concentration in the surface sediment diminish with distance towards the estuary. Secondly, the lead concentrations on surface sediments are much lower in the

Table 4.3

Lead Concentration in Surface Sediments of the Avon-Heathcote River System.

Site No.	Km	Site Description	Lead Concentration
Heathcote River			
1	7.3	10m Downstream from Opawa Rd. Bridge	73 (62)
2	6.7	Foot Bridge, Opposite Sheldon St.	320 (232)
3	6.0	Near Radley St. Bridge	336 (102)
4	5.4	Catherine St. Foot Bridge	321 (21)
5	4.9	Intersection of Cumnor Tce. and Chichester St.	363 (30)
6	4.5	Garland Rd. Bridge	606 (349)
7	4.0	Corner of King Edward Tce. and Bamford St.	311 (61)
8	3.6	Corner of Broad St. and Stanton St.	133 (68)
9	2.8	Corner of Barton St. and Long St.	208 (102)
10	2.4	Lyttelton Tunnel Rd. Bridge	141 (59)
11	1.9	Opposite Fire Station on Ferry Rd.	151 (11)
12	0.1	Ferry Rd. and Bridle Path Rd. Bridge	93 (10)

Table 4.3 cont.

Site No.	Km	Site Description	Lead Concentration
Avon River			
13	5.1	Avondale Rd. Bridge	189 (9)
14	3.9	Opposite Cowles St.	78 (5)
15	3.5	Wainoni Rd. Bridge	31 (2)
16	2.8	Opposite Baker St.	71 (6)
17	2.1	100m Downstream of Pages Rd. Bridge	84 (9)
18	1.5	Opposite Evans Ave.	38 (3)
19	0.8	Opposite Corner of Falcon St. and Kibblewhite St.	39 (3)
20	0.2	Dyers Rd. Bridge	17 (3)
Estuary			
21		Pleasant Point Domain (near Jetty)	24.2 (0.8)
22		200m Off Shore from Penguin St.	8.0 (0.6)
23		100m Off Shore from Beachville Rd.	10.5 (0.4)

Table 4.3 cont.

Notes: (1) Lead Concentration in $\mu g Pb g^{-1}$ (dry weight).

(2) Determinations are on 3-5 samples at each site.

(3) Sites are as in Figure 4.1.

(4) Km are taken as distance from the Estuary.

(5) Values are mean with standard deviation in brackets.

Figure 4.2

Graph Showing Change in Lead Concentration in Sediment with Distance from the Mouth of the Avon River.

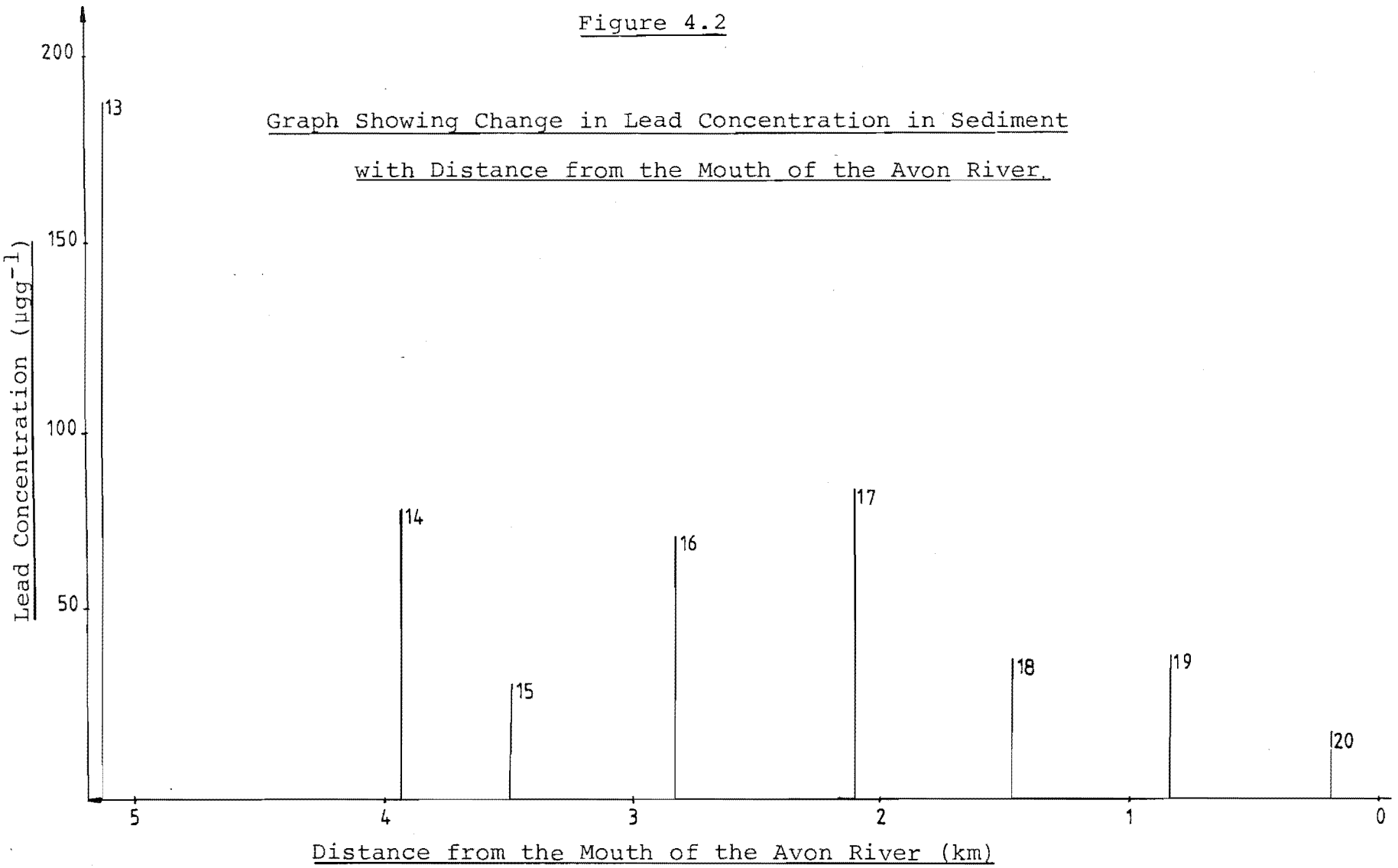
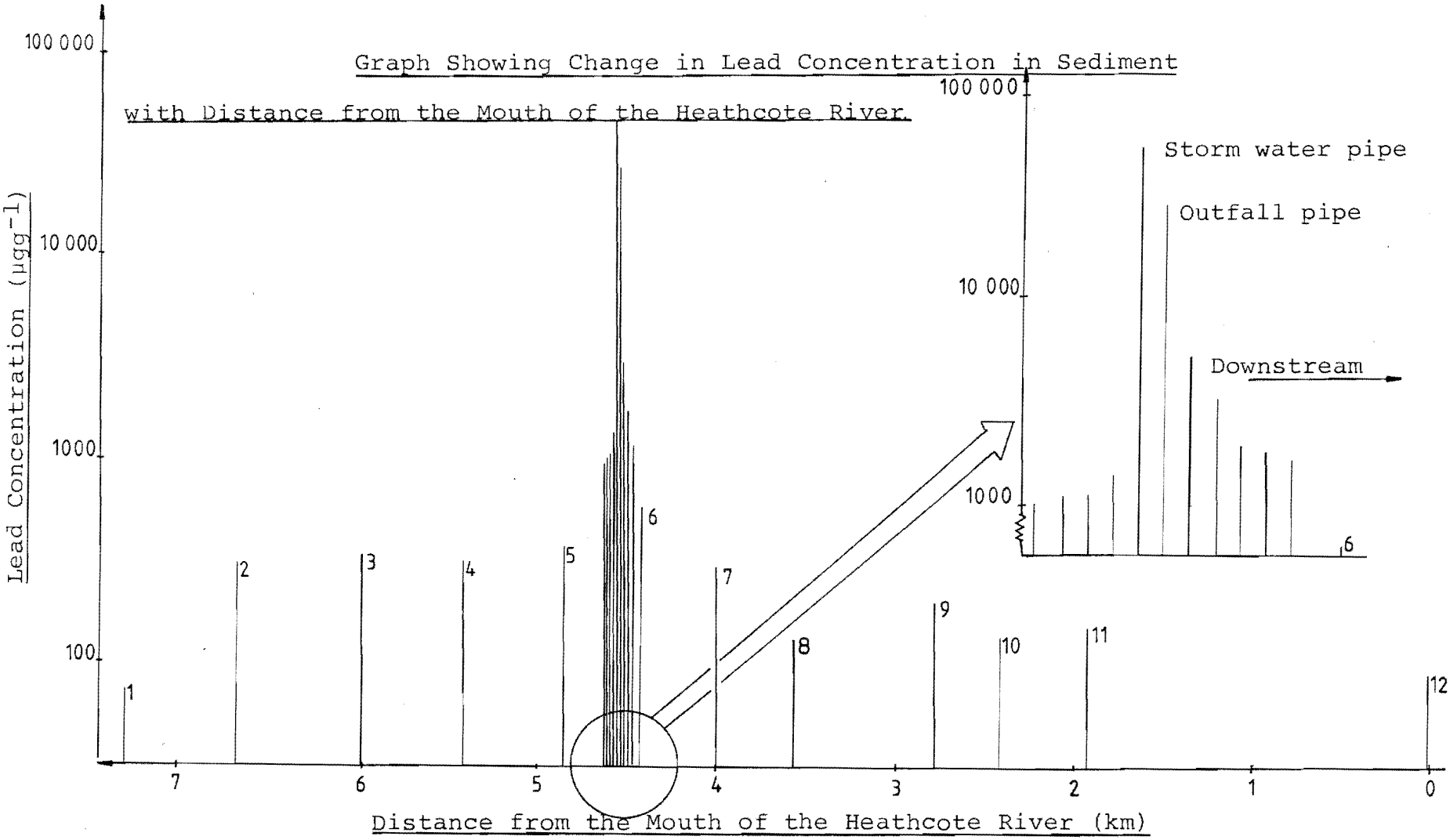


Figure 4.3


Graph Showing Change in Lead Concentration in Sediment
with Distance from the Mouth of the Heathcote River.
Lead Concentration (µgg⁻¹)
100 000
10 000
1000
100
Storm water pipe
Outfall pipe
Downstream
1
2
3
4
5
6
7
8
9
10
11
12
7
6
5
4
3
2
1
0
Distance from the Mouth of the Heathcote River (km)

estuary than in the upper reaches of the rivers (see Figures 4.2, 4.3). This is probably due to either dilution, a result of the mixing of sea water and the river borne material, or by constant erosion of sediment by the tidal passage within the estuary and the lower reaches of the river or relatively firm "fixing" of lead by the sediments in the river and low mobility of the sediments. Probably all three factors play a part. This decrease with distance from the source of pollution and the dilutant effect of tidal flow has been noted previously (16, 20). Muller and Forstner (16) argued that the lowering of heavy metal concentration towards the mouth of the Elbe River was due to the dilution of the highly polluted sediments with less polluted sediment downstream by mixing, and not due to the mobilisation of heavy metals from the sediments into some soluble form which was washed down by the river.

A third point of note in the survey is the rise in lead concentration immediately downstream of the Pages Road Bridge, (Site 17), on the Avon River. This is believed to be due to the fact that a large amount of storm water from the suburb of New Brighton goes into the river. Storm water carries road dust with it and this may be the cause of the rise in lead concentration, as the area prior to this (Site 15) through which the river flows does not have a high population density near it. Even further up river, where the river flows through urban Christchurch, the level of lead increases (Site 13) to 189$\mu gPbg^{-1}$. Bloom and Ayling (19) noted this effect on a much greater scale on the Derwent River near Hobart, Australia. These results do suggest a reasonable immobility of the lead contaminated

sediments.

At Site 8 on the Heathcote River, a drop in the lead concentration is observed. This may be more apparent than real, as the sediment collected at the sampling site was sandy while for all the other sites the material was considerably more muddy in composition. More mud is indicative of a greater clay fraction and it appears that heavy metals are found in greater concentrations in the clay fraction of the sediment (18).

The highest concentration of lead was found near the Garlands Road Bridge (Site 6), which is just downstream from a battery factory. In order to look at this area more closely, samples were taken every 20 metres for 220 metres upstream from Site 6. The results of this study are given in Table 4.4 and are shown in an expanded diagram in Figure 4.3. The outfall pipe from the battery factory is at 120 metres upstream from the Garlands Road Bridge and at 140 metres upstream of the Garlands Road Bridge is a storm water drain that flows from a small smelter within the battery factory's grounds.

These observations of lead concentration are in general agreement with those of Tan (2), except that his peak level near the battery factory was 27000$\mu gPbg^{-1}$. He also observed the fall off in lead concentrations as the rivers approached the estuary. While general agreement with Tan's (2) work on estuary sediment occurs, he did not find the surface sediment higher at Pleasant Point (Site 21). The Pleasant Point site is of interest in that Macpherson (1) has postulated that it was the only area of the estuary where sediment is being built up. It is

Table 4.4

Lead Concentrations in Surface Sediments of the Heathcote River near the Battery Factory.

Distance (metre)[1]	Lead Concentration[2]
20	1650±100
40	1850±100
60	1900±100
80	3200±100
100	5100±200
120[3]	28 300±800
140[4]	54 900±1600
160	1400±100
180	1100±100
200	1100±100
220	1000±100

Notes: (1) Distance is defined as metres upstream from sample site (6), Garlands Rd. Bridge.

Table 4.4 cont.

Notes: (2) Lead concentration is in $\mu gPbg^{-1}$ of sediment on a dry weight basis.

(3) Output from battery factory.

(4) Output from storm water drain from battery factory smelter.

(5) Values are mean±error

certainly the one area in the estuary where the sediment is a lot more "muddy" in character compared to the sandy nature of the rest of the estuary. The level of lead found in this area is greater than the other two areas sampled.

Macpherson (1) proposed that in the early settlement of Christchurch, there was an appreciable build up of mud within the Avon-Heathcote Estuary. Later, urbanisation of Christchurch, which increased the catchment area of the estuary and speed-up of the run off from the catchment area, caused this mud to be washed out to sea and that the tidal compartment is now still increasing in size. However, there is a residue of the early mud in the Pleasant Point area and he believed that heavy metals may be accumulated here, as the area did not suffer from erosion like the rest of the estuary. At 0.5 cm depth the lead concentration was $24.2 \pm 0.8 \mu gPbg^{-1}$ but at 4 cm below the surface the lead concentration had dropped to $15.2 \pm 0.7 \mu gPbg^{-1}$ and at 18 cm below the surface the lead concentration was now only $8.6 \mu gPbg^{-1}$. This trend would support Macpherson's (1) beliefs and appears to be the only site within the estuary where accumulation is occurring.

The higher levels of lead found in Heathcote River sediments are due to the high level of industrial activity along its banks. In comparison, much lower levels of lead are found in Avon River sediments because the major source of lead for this river is mainly urban run off which carries lead from the streets of Christchurch.

4.3.2 Lead Concentration in Profiles of Sediments Taken near the Battery Factory.

Because of the high concentrations of lead near the battery factory, a series of six sediment profiles were taken near the factory. The profiles were taken from the following sites:

(I) approximately 1 metre upstream of the storm water drain from the factory's smelter.
(II) approximately 1 metre downstream of the storm water drain from the factory's smelter.
(III) on the opposite side of the river from (II).
(IV) 50 metres upstream from (II).
(V) 50 metres downstream from (II).
(VI) within 1 metre of the outfall pipe from the battery factory's waste pipe.

A description of the profile from each site is given in Figure 4.4. The sediment is predominantly mud, but as these profiles are obtained near the river bank, there is a large amount of decaying vegetation from the trees that grow along the river bank. Also, there is evidence to suggest that garden rubbish and some fire ash had been dumped into the river near the sampling sites; and some traces of coke were found in some of the profiles. The results of the lead analysis on these sediment profiles are given in Table 4.5 and graphically in Figure 4.5.

From the results, the first observation that can be made, is the high degree of localisation of the lead

Figure 4.4

Profile Descriptions

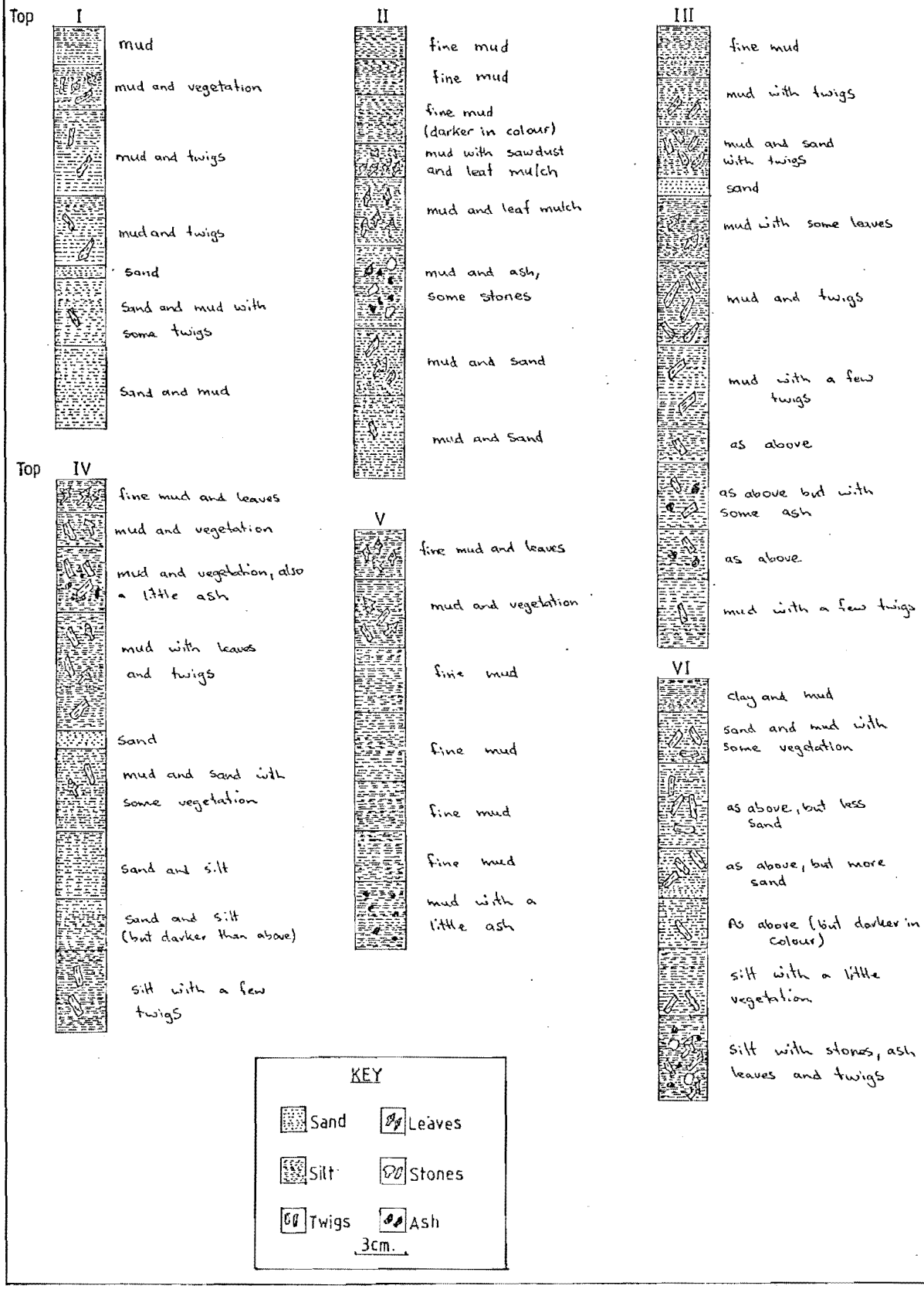


Table 4.5

Lead Concentrations in Sediment Profiles obtained near the Battery Factory on the Heathcote River.

Profile I		II		III		IV		V		VI	
Depth	$[Pb^{2+}]$	Depth	$[Pb^{2+}]$	Depth	$[Pb^{2+}]$	Depth	$[Pb^{2+}]$	Depth	$[Pb^{2+}]$	Depth	$[Pb^{2+}]$
2	3100±60	2	80 000±2000	3	480±60	2	720±20	3	740±20	2	4800±120
5	2900±100	4	58 000±2000	6	440±10	4	810±40	7	520±20	5	2700±100
10	3900±100	7	82 000±3000	9	300±10	8	730±20	11	600±20	10	2300±100
14	1600±100	9	87 000±3000	10	150±10	15	650±20	15	730±30	13	2600±100
15	200±10	13	76 000±3000	14	500±40	16	180±5	18	720±40	16	4800±100
19	160±10	18	54 000±2000	19	280±10	21	90±5	21	1360±60	20	3600±100
24	130±10	22	800±40	24	21±3	25	130±5	25	790±50	25	8400±3
		27	2300±100	26	22±3	28	150±10				
				30	17±3	33	30±4				
				33	22±3						
				37	20±3						

Table 4.5 cont.

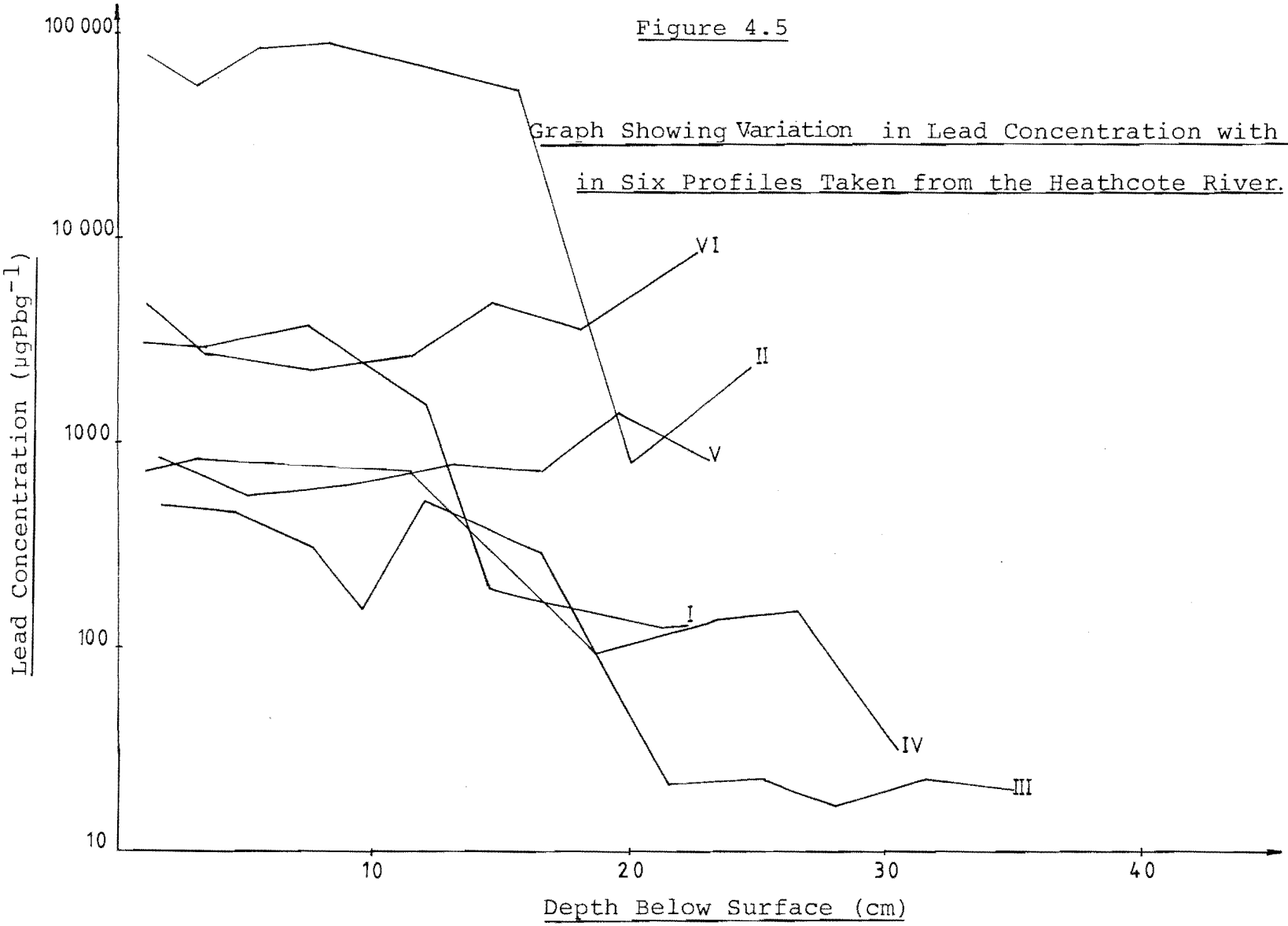
Notes: (1) The value for depth is in cm and is the value at the bottom of the slice of profile taken as the sample.

(2) Values are mean±error.

(3) Lead concentration in $\mu g Pb g^{-1}$ dry weight.

Figure 4.5

Graph Showing Variation in Lead Concentration with Depth in Six Profiles Taken from the Heathcote River.



in sediment close to the point of input. An illustration of this, is that the surface lead concentration at the output site (profile II) is twenty times higher than a site 2 metres upstream (profile I) and is 160 times higher than a site 8 metres just across the river (profile III). In all profiles, except V and VI, the concentration of lead falls off with increasing depth into the sediment. Profile VI is close to the site of an old outfall from the battery factory, and it is understood that higher levels of lead (than at present) were dumped through this outfall. Profile V is downstream and the constant level of lead in the profile probably reflects the slow movement of polluted sediments down the river.

In the zones of the profile where there is a high sand content, the lead concentration tends to be lower, and in areas where there is a high amount of decaying organic matter then the lead concentration tends to be higher. Several authors have noted that the heavy metals tend to be concentrated in areas of high organic content and where the sediment particle size is small (17, 18, 21).

Although the lead concentration was high at the site of output from the battery factory's smelter (ranging from 5.4 to 8.7% over the depth of 2-18 cms), similar levels have been found elsewhere. In the Sörfjord, West Norway, lead concentrations of 10% were found near a lead smelter (21), in Derwent Estuary levels of 4.2% were found near a wharf used for loading zinc ores (19). Concentrations of lead in the 1000-10 000$\mu gPbg^{-1}$ have been found on surface sediments in highly polluted rivers e.g. the River Vesdre (22), the River Biala Przemsza (23) and the Blies River (24).

4.3.3 Variation of pH with Depth in Heathcote River Sediments.

In order to see if there was any systematic change in the pH of the sediment with depth below the surface, samples from the top, middle and bottom of each profile (see Figure 4.4) were measured for pH. Sediment (2g) was mixed with 5mL of either distilled water or 0.01M $CaCl_2$ solution. The calcium chloride solution was used in an attempt to simulate the ionic strength of a solution similar to that which the sediment is normally resident in.

The results of the determination of pH in sediment profiles is given in Table 4.6. In general, there is little variation down the profile, except for profile II, which shows a marked decrease in pH down the profile. For all other profiles the pH remains relatively steady down the column, or shows a tendency to a slight increase down the column. Profile II is the sample taken beneath the outfall pipe from the battery factory, and the presence of large amounts of lead salts may affect the pH of the sediments, as may any acid outfall from the factory.

Two samples of the discharge water from the factory were obtained when the discharge contained lead wastes, the pH was 6.2 ± 0.1, and on another occasion when only water was being discharged the pH was 6.7 ± 0.1. It is possible that hydrolysis of lead salts would occur giving slightly acid solutions. River water samples tested had a mean $pH = 6.9 \pm 0.1$.

Table 4.6

Variation in pH with Depth in Sediment Profiles taken from the Heathcote River.

Profile	Depth	0.01M $CaCl_2$	Distilled Water
I	0-2	4.9	5.4
	10-14	5.7	5.9
	19-24	5.5	5.7
II	0-2	6.0	6.2
	9-13	5.7	5.9
	18-22	4.9	5.4
III	0-2	5.6	5.8
	10-14	5.6	5.8
	19-24	5.8	6.1
IV	0-2	5.9	6.2
	8-15	6.0	6.3
	21-25	5.5	5.8
V	0-3	5.6	5.9

Table 4.6 cont.

Profile	Depth	0.01M $CaCl_2$	Distilled Water
V	7-11	5.3	5.7
	21-25	5.9	6.3
VI	0-2	5.4	6.0
	10-13	5.5	5.9
	20-25	5.8	6.1

Notes: (1) Profiles the same as in Figure 4.4.

(2) 2g of sediment and 5 mL of suspending medium, left overnight to equilibriate.

(3) Depth values are in cm below the surface.

4.3.4 Investigation of the Geochemistry of Lead in the Heathcote River Sediments.

Because of the very high levels of lead from profile II, it was decided to see if it was possible to concentrate the lead compounds in the sediment and then to investigate the chemical form of the lead compounds present. The first step in this process was to attempt to separate the lead containing fraction from the silicates that made up the rest of the sediment.

The method chosen to separate lead minerals from the silicates of the sediment was densiometric separation using tetrabromoethane (density 2.95gcm^{-3}). The material with a density of 2.96gcm^{-3} or greater was then collected, washed with ethanol and dried. Material of a magnetic nature was then removed with the help of a permanent magnet and kept separate. The three fractions were then analysed and the results of the analyses as well as the percentage of each fraction are given in Table 4.7.

From the results it is apparent that the non-magnetic fraction of density greater than 2.96gcm^{-3} contained the highest levels of lead, and the concentrations were now sufficient to allow for analysis of this fraction for specific lead compounds. This was done initially using X-ray powder diffraction to give an indication of which crystalline compounds were present. The results of this investigation are given in Table 4.8, and a tentative order of the abundance of the compounds is given with the compound producing the strongest diffraction lines listed first, and the one giving the weakest lines last.

Table 4.7

Results of Densiometric Separation on Profile II from the Heathcote River.

Depth	Density<2.95gcm^{-3}		Density>2.95gcm^{-3}				Weight Loss
			Magnetic		Non-magnetic		During
	Weight(g)	[Pb^{2+}] %	Weight(g)	[Pb^{2+}] %	Weight(g)	[Pb^{2+}] %	Separation(%)
0-2	8.08	5.4	0.24	14.4	1.50	56.6	1.8
2-4	8.97	3.5	0.11	12.2	0.76	45.7	1.6
4-7		5.1	0.11	9.7	0.92	41.7	
7-9	8.64	6.1	0.17	17.9	0.75	44.9	4.4
9-13	8.90	7.7	0.03	11.3	0.73	37.9	3.4
13-18	9.17	3.7	0.05	16.4	0.61	34.0	1.7
18-22	9.80	0.22	0.015	3.0	0.07	18.2	1.2
22-27	10.0		1.2mg		3.2mg		

Notes: (1) Depth is in cm below the surface.

(2) Lead concentration is in %W/W.

Table 4.7 cont.

Notes: (3) Initial weight of each sample was 10.00g.

(4) For the 4-7 sample some of the material was spilt during transfer.

(5) For the 22-27 sample fractions were too small for analysis.

Table 4.8

Results of Analysis by Powder X-Ray Diffraction on the Sediment of Profile II from the Heathcote River (Fraction With D>2.96gcm^{-3} and non-magnetic)

Depth (cm)	Lead Compounds Present in Profile
0-2	$PbCO_3$, $PbSO_4$, Pb
2-4	$PbCO_3$, $PbSO_4$, Pb, PbS
4-7	$PbCO_3$, $PbSO_4$, Pb, PbS
7-9	$PbCO_3$, $PbSO_4$, Pb
9-13	
13-18	PbS, $PbSO_4$, Pb
22-27	Pb, PbS, $PbSO_4$, $PbCO_3$

A sample of fly ash obtained from the smelter at the battery factory was similarly analysed and found to contain just lead metal and lead sulphate. Quantification of the amounts of each compound using infrared spectroscopy, was not possible as only lead sulphate and lead carbonate had absorbance within the working range of the machine, and the remaining clay and organic material in the sample produced serious background curvature in the IR spectra. Therefore differential thermal analysis (DTA) was used. Plots of temperature differential as a function of temperature for the sediment samples are given in Figure 4.6 and for the three lead compounds in Figure 4.7.

For lead carbonate, heated in still air, Worne and Balysis (25) believed that three reactions were involved in the thermal decomposition:

Figure 4.6

Differential Thermal Plots for Profile II from the Heathcote River.

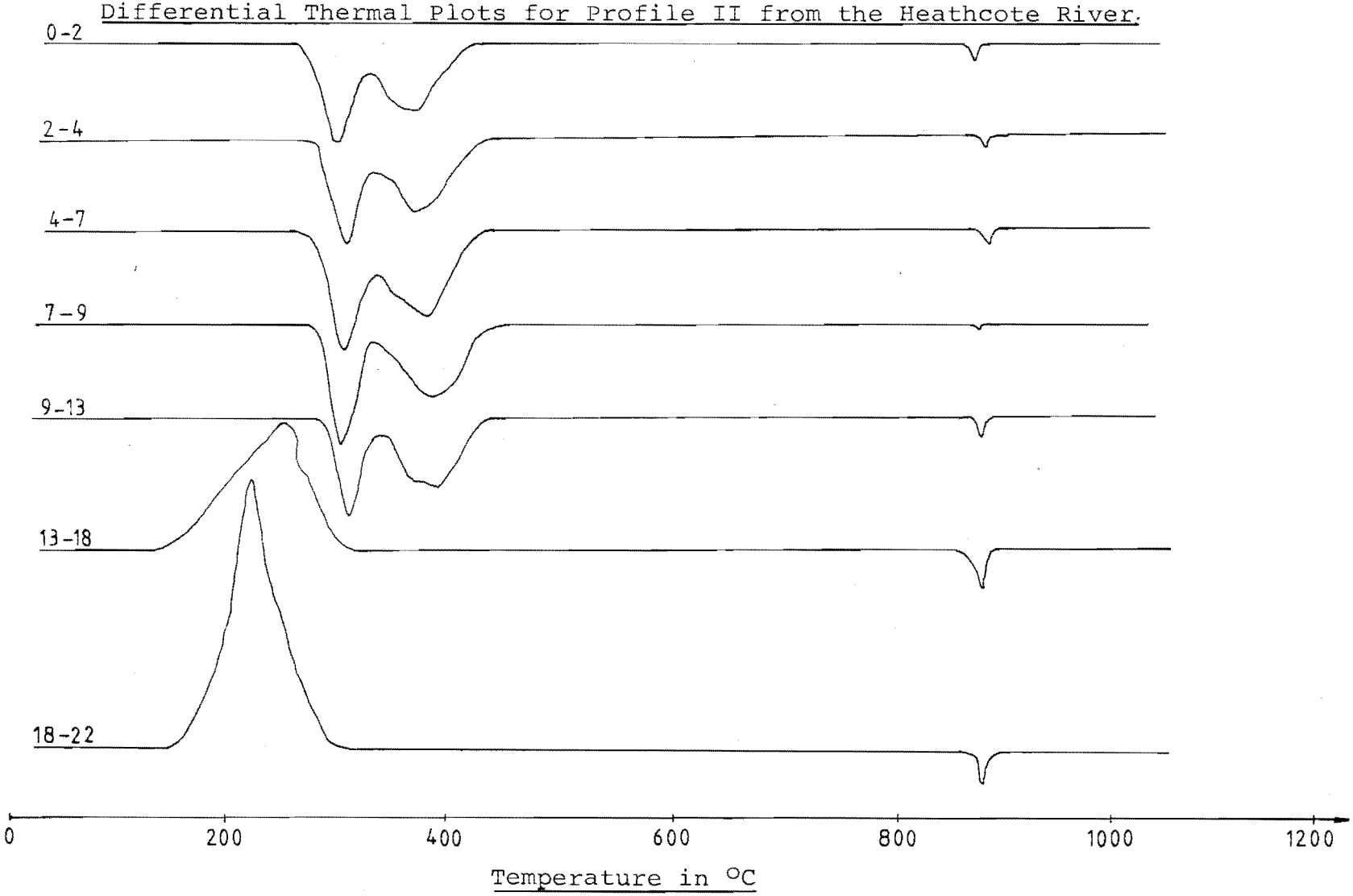
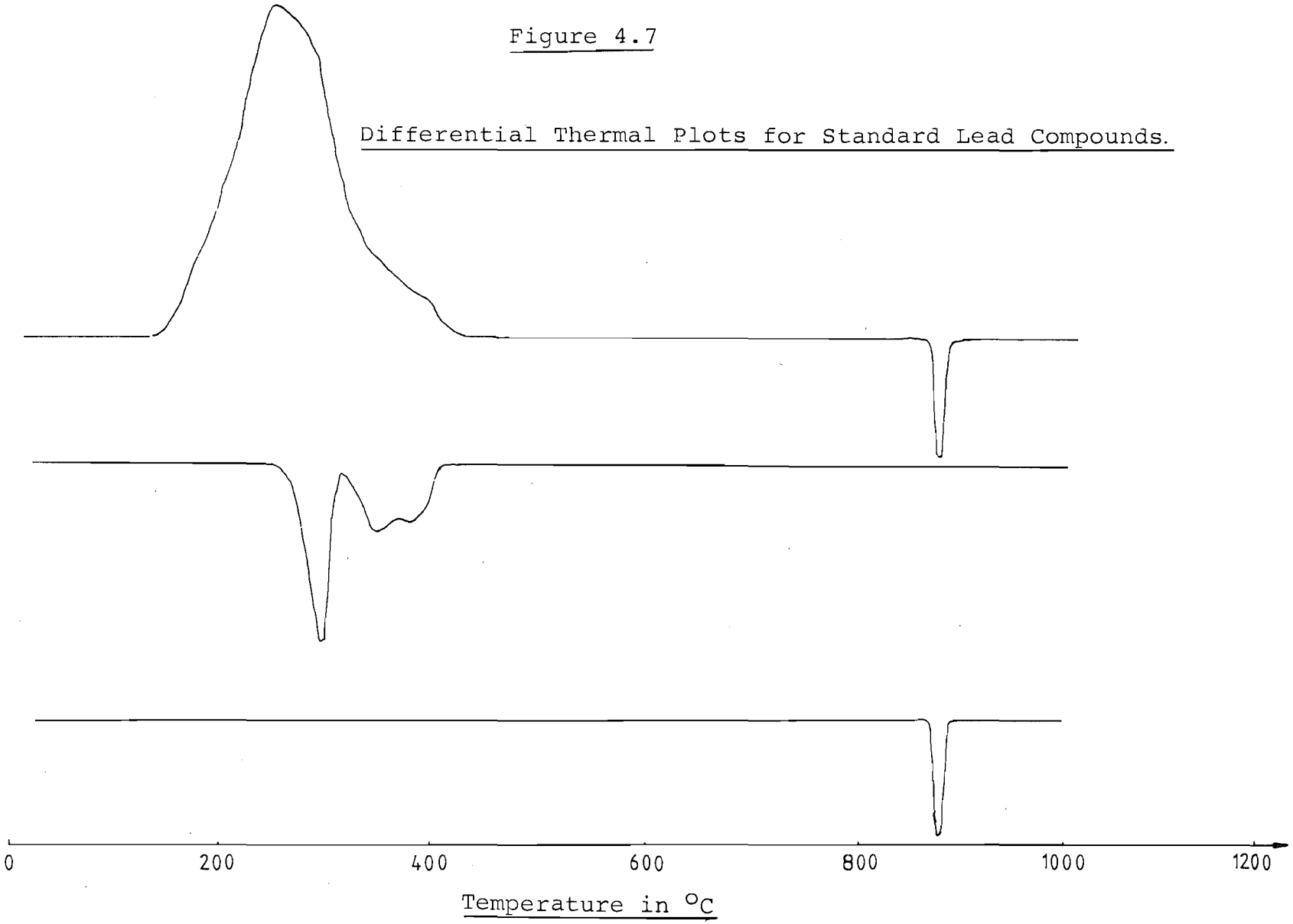


Figure 4.7

Differential Thermal Plots for Standard Lead Compounds.



$$2PbCO_3 \xrightarrow{340^{\circ}C} PbO.PbCO_3+CO_2$$

$$3(PbO.PbCO_3) \xrightarrow{390^{\circ}C} 2(2PbO.PbCO_3)+CO_2$$

$$2PbO.PbCO_3 \xrightarrow{440^{\circ}C} 3PbO+CO_2$$

The sharp peak in the lead sulphate and lead sulphide traces at approximately 850°C is due to a rhombic to monoclinic inversion (26). The strong exothermic peak for lead sulphide is an oxidative peak due to the formation of lead sulphate from lead sulphide.

$$PbS + 2O_2 \xrightarrow{200-300^{\circ}C} PbSO_4$$

The shape of the lead sulphide curve is affected by the size of the lead sulphide grains and the ease of entry for oxygen to the sample (26).

It was possible to calculate the amount of each lead compound present by the use of calibration curves using pure lead compounds. Then the percentage of lead tied up in each compund at a particular depth in the profile can be estimated. These results are given in Table 4.9. For the three compounds the percentage of the total lead in each compound is given in Table 4.10 and shown diagrammatically in Figure 4.8. From these results it is possible to calculate the concentration of lead in each sample due to the three lead compounds found and compare this with the total concentration of lead in each sample. However, the large errors associated with the DTA analysis are such, that this really does not give an accurate measure of the amount of lead present in the samples as lead metal and other forms of lead such as organically bound or sorbed.

Table 4.9

Quantity of Lead Compounds in Profile II Sample by DTA.

Depth (cm)	Sample (mg)	$PbCO_3$ (mg)	$PbSO_4$ (mg)	PbS (mg)
0-2	40	18±2	5±1	
2-4	40	18±2	4±1	
4-7	40	20±2	4±1	
7-9	40	19±2	2±1	
9-13	40	16±2	5±1	
13-18	40	1.9±0.5	8±1	4.2±0.5
18-22	20		0.5±0.5	3.5±0.5

Notes: (1) Depth is in cm below the surface.

(2) Value given is result±error.

Table 4.10

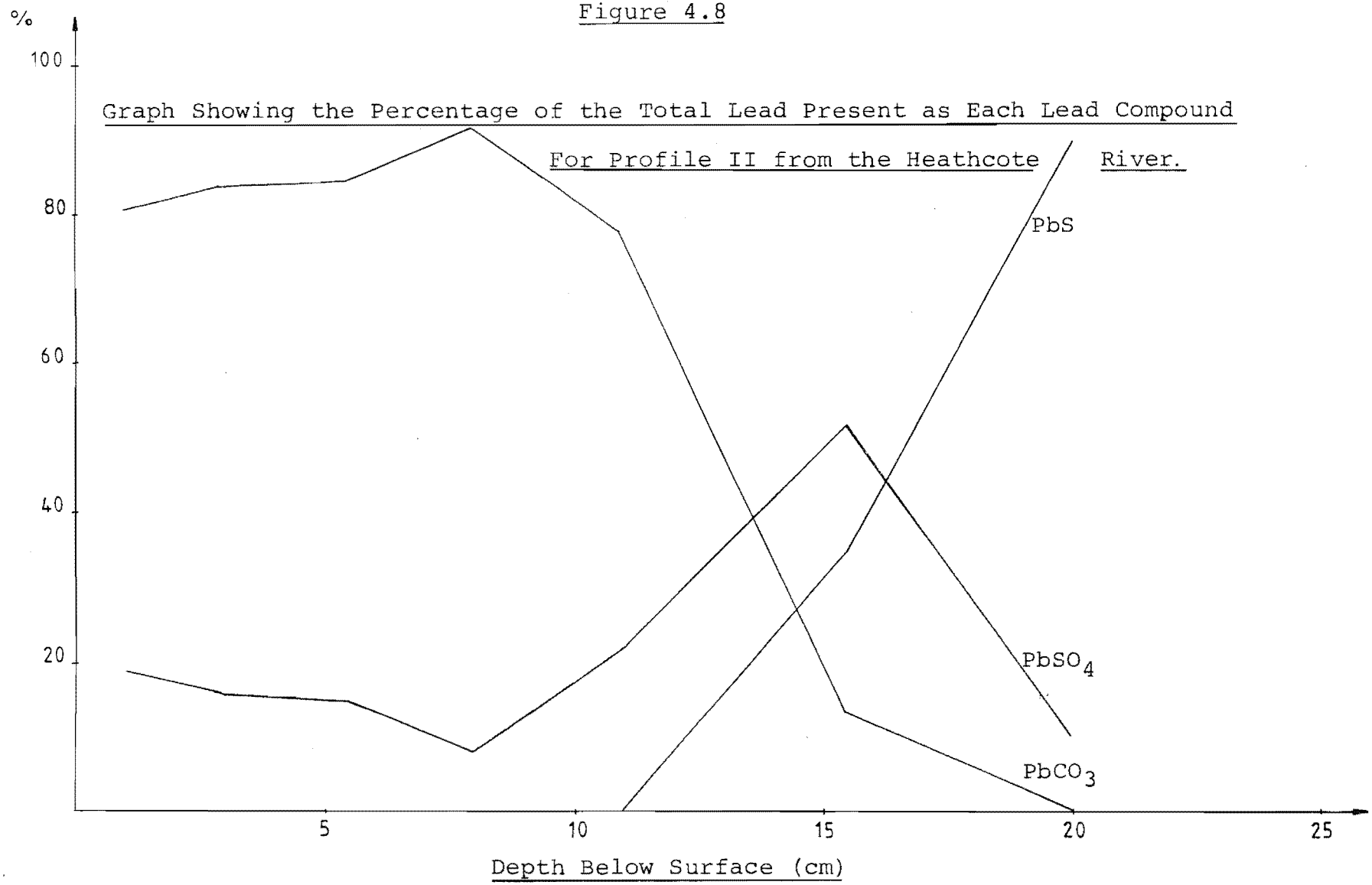
Percentage of the Lead in each Compound in Profile II.

Depth (cm)	$PbCO_3$ (%)	$PbSO_4$ (%)	PbS (%)
0-2	81±19	19±3	
2-4	84±21	16±6	
4-7	85±21	15±6	
7-9	92±23	8±5	
9-13	78±21	22±8	
13-18	13±5	52±23	35±9
18-22		10±8	90±16

Notes: (1) Depth is in cm below the surface.

(2) Value given is result±error.

Figure 4.8



Graph Showing the Percentage of the Total Lead Present as Each Lead Compound For Profile II from the Heathcote River.

However, the results are suggestive that the biggest proportion of the lead in the sediment is accounted for by the three compounds $PbSO_4$, $PbCO_3$, and PbS. (See Table 4.11).

In order to explain the changing pattern of lead compounds in the profile, two factors have to be considered. Firstly, the pH of the sediment decreases with increasing depth below the surface, (see Table 4.6 and Figure 4.9) and this would favour the conversion of carbonate to sulphate. Bryne (27) suggests from UV spectroscopy evidence, that $PbCO_3$ is the stable species in sea water. Sipos et al (28) also believe that the stable species is carbonate or $Pb(CO_3)_2^{2-}$ and determined this by voltammetric analysis. While it would appear that carbonate is the stable species at neutral pH, as the sediment becomes more acidic then the sulphate will become more prominent. As a consequence of this change of pH with depth, conversion of the carbonate into the more soluble sulphate ($K_{sp}^{298}PbCO_3=6x10^{-14}$, $K_{sp}^{298}PbSO_4=1.7x10^{-8}$) could account for the decrease in lead concentration between 13 and 18 cm below the surface as $PbSO_4$ is more readily leached out.

Also, in going down a sediment profile, the environment, in general, becomes more reducing. This would favour the conversion of sulphates to sulphides at lower depths, as observed. It has also been suggested (29, 30) that heavy metal ions which are sorbed onto organic sulphur groups in decaying material eventually form heavy metal sulphides. As the sulphide is the least soluble of the lead salts found, ($K_{sp}^{298}PbS=8.4x10^{-28}$) then it would be expected that lead concentrations would start to rise at

Table 4.11

Lead Concentration in Profile Sample by AAS and DTA.

Depth (cm)	Lead Concentration	
	AAS	DTA
0-2	56.6	45±6
2-4	45.7	42±6
4-7	41.7	46±6
7-9	44.9	38±5
9-13	37.9	39±6
13-18	34.0	27±5
18-22	18.2	16±5

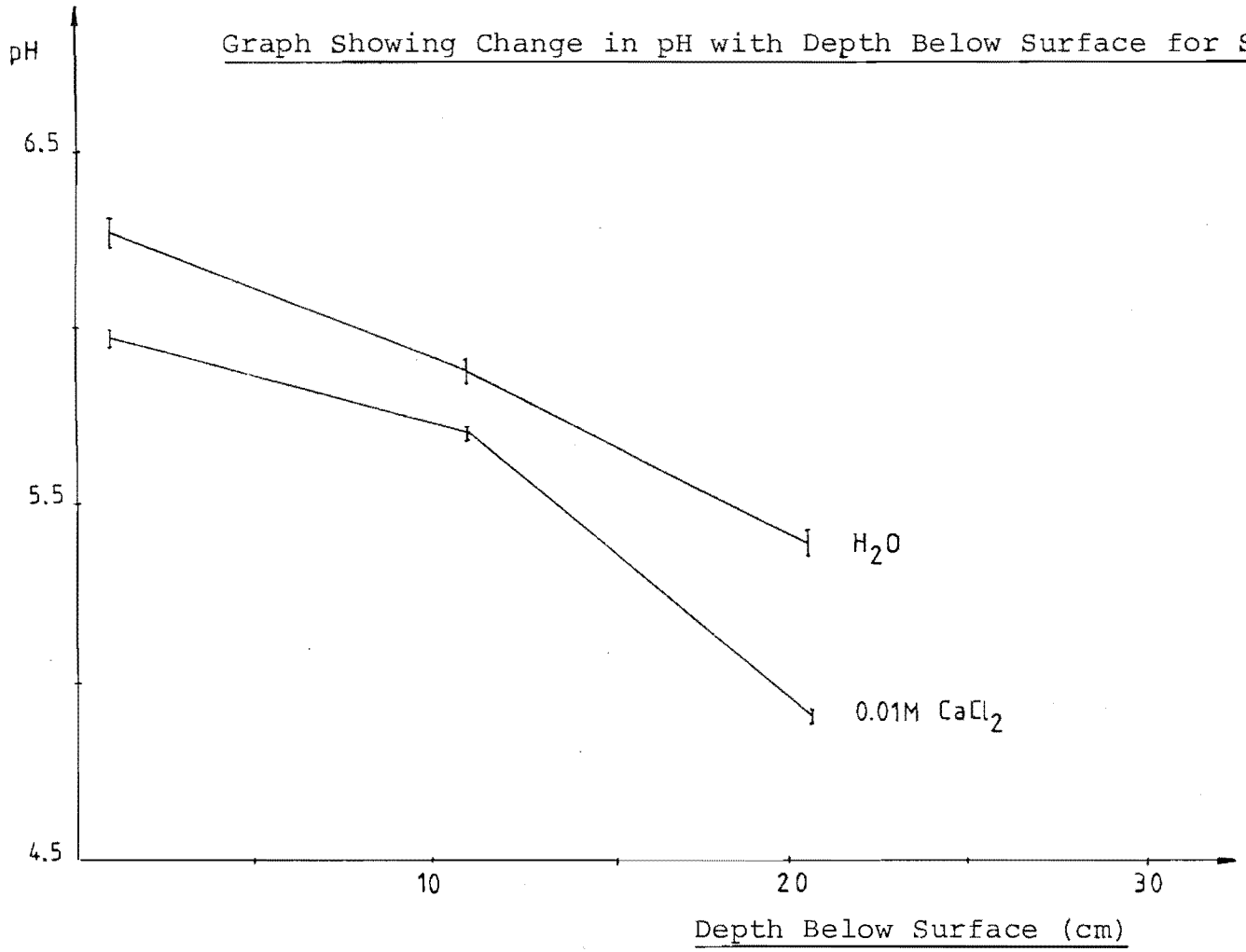
Notes: (1) Depth in cm below the surface.

(2) Lead Concentration is in %W/W

(3) Values are mean±error.

Figure 4.9

Graph Showing Change in pH with Depth Below Surface for Sediment Profile II



lower depths as lead sulphide is formed and accumulates.

To check to see if oven drying of the sediment had an influence on the chemical forms of lead in the profile, a second profile from the same site was just air dried. It showed the same distribution of lead compounds as indicated by the X-ray powder diffraction patterns.

4.3.5 Sorption of Lead Ions onto Sediments.

Since some lead is found in the low density fraction of the sediment it is possible that some of this is sorbed onto the sediment. Three sorption sites are possible viz. cation exchange sites on clays, ferro-manganous oxides or by bonding to organic matter. To investigate the sorption of lead ions onto sediment, a sample of sediment obtained from the upper reaches of the Heathcote River (RC) but with a relatively low level of lead ($81 \mu g Pb g^{-1}$) was used.

Because of the need to hold pH constant during the sorption studies, and because lead precipitates at pH's close to those of natural waters, the experiments were carried out in a 0.02M acetate buffer. The first experiments were carried out to find the time to reach equilibrium between the lead ions in solution and those sorbed onto the sediments. This study was carried out using two different initial quantities of lead added to the solution. The results given in Table 4.12 and Figure 4.10 show that equilibrium was reached within 24 hours.

In order to study the equilibrium between the quantity of lead sorbed onto the sediment and the lead concentration in solutions, twelve flasks, each containing 1g of sediment

Table 4.12

Time to Establish Equilibrium for Lead Added to Sediment (RC) from the Heathcote River.

	Lead Sorbed $\mu gPbg^{-1}$ of Sediment	
Time	A	B
5	2050±100	3830±100
15	2390±100	5220±100
45	2290±100	5210±100
105	2660±100	5240±100
300	2760±150	5270±100
480	2490±200	5230±200
1410	2550±100	5230±100

Notes: (1) Time in minutes, with an error of one minute.

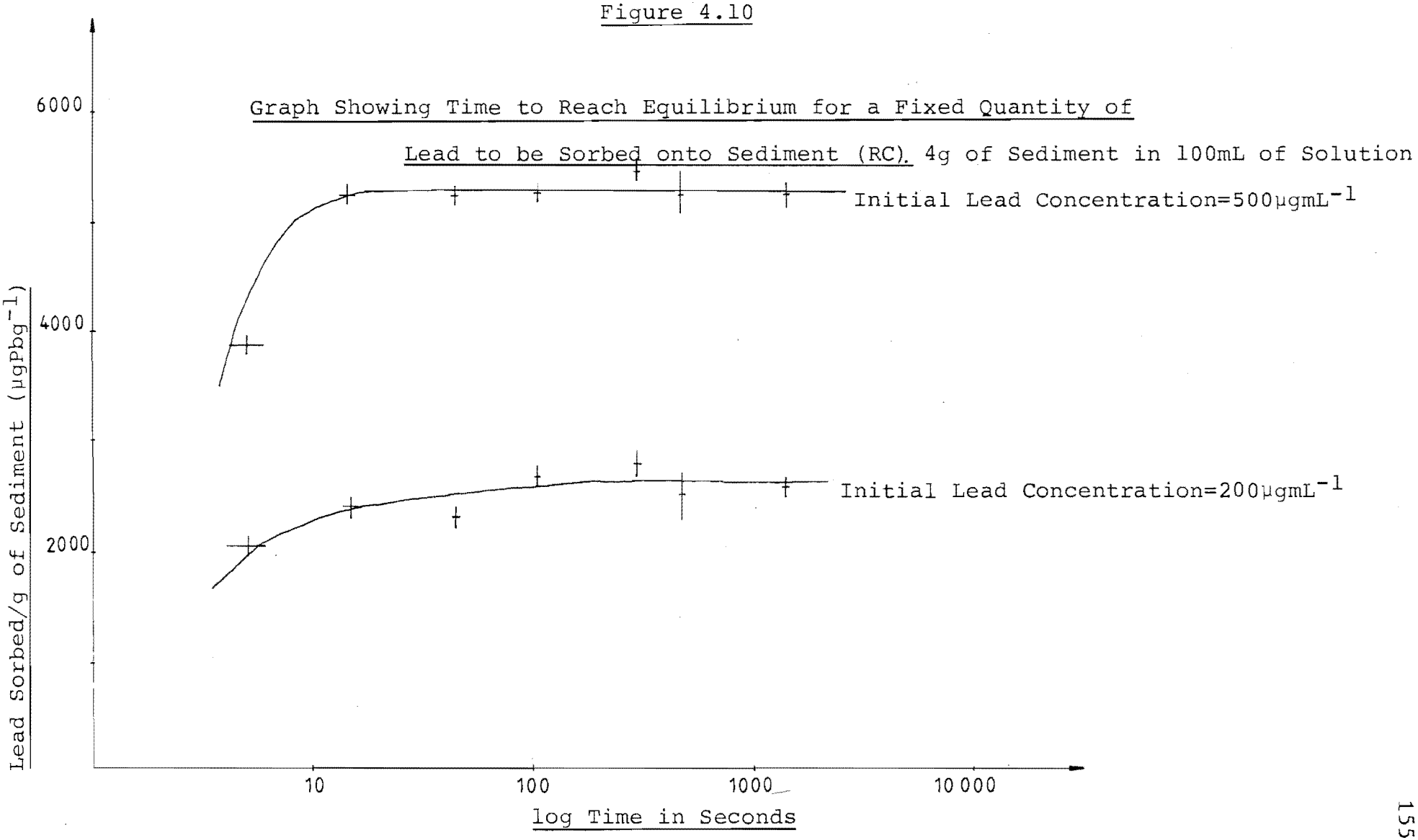
(2) Amount of sediment used in experiment was 4.00g.

(3) A=20 000 µgPb added to solution.

(4) B=50 000 µgPb added to solution.

(5) Values are mean±error.

Figure 4.10


Graph Showing Time to Reach Equilibrium for a Fixed Quantity of
Lead to be Sorbed onto Sediment (RC). 4g of Sediment in 100mL of Solution
Initial Lead Concentration=500µgmL-1
Initial Lead Concentration=200µgmL-1
Lead Sorbed/g of Sediment (µgPbg-1)
6000
4000
2000
10
100
1000
10 000
log Time in Seconds

and 25mL of solution, were set up in a shaking rack and placed into a thermostat bath maintained at 25±0.5°C, for 24 hours. The quantity of lead added to each flask was varied from between 0.5 to 20mL of 1000μgPbmL^{-1} solution. All flasks also had acetic acid/sodium acetate buffer (pH=4.74) at 0.02M concentrations. After 24 hours 2mL was removed from each flask, centrifuged to cause the sediment to settle to the bottom and the supernatent fluid was analysed by flame atomisation-AAS.

The results of three separate experiments are given in Table 4.13 and Figure 4.11. It is possible from these results to construct a Langmir Isotherm by plotting the ratio of equilibrium lead concentration to the quantity of lead sorbed per gram of sediment as a fraction of the equilibrium concentration of lead. For run II for which more data is available, the Langmir Isotherm was plotted (see Figure 4.12). The plot was not a straight line as would have been predicted if the relationship held. Griffin and Au (31) also found curvature of the Langmir Isotherm, and suggested that this was due to the presence of competing ions. As lead is sorbed onto clay, other ions would be desorbed which could later (as their concentration became significant) compete with lead for sorbing onto the clay.

In the paper of Salim and Cooksey (32) they found that the three factors which affected lead sorption onto river mud, were the quantity of organic matter, as this had a greater sorbing capability than the inorganic component, the pH at which sorption occurs, and the size of the particles of inorganic matter. In order to investigate the effects of the clay type, percentage of

Table 4.13

Sorption of Lead onto Heathcote River Sediment (RC).

	Run I		Run II	
Initial Lead Concentration (μgmL^{-1})	Lead Concentration at Equilibrium (μgmL^{-1})	Lead Sorbed on Sediment ($\mu gPbg^{-1}$)	Lead Concentration at Equilibrium (μgmL^{-1})	Lead Sorbed on Sediment ($\mu gPbg^{-1}$)
30±0.5	1.8±0.1	710±20	1.8±0.1	700±20
60±2	6.2±0.2	1290±60	7.5±0.3	1300±60
120±3	22.7±0.9	2380±90	17.1±0.7	2550±80
180±3	50±2	3300±130	28.2±1.2	3000±100
240±3	85±3	3880±150	49±1.3	4880±110
300±3	123±6	4270±230	76±4	5470±180
360±4	160±8	4880±300	102±5	6640±230
460±4	220±10	5770±350	140±6	6840±250
520±4	293±14	6300±450	158±7	8600±300
600±5	350±14	6200±500	208±9	9900±300

Table 4.13 cont.

Run I		Run II	
Initial Lead Concentration (μgmL^{-1})	Lead Concentration at Equilibrium (μgmL^{-1})	Lead Concentration at Equilibrium (μgmL^{-1})	Lead Sorbed on Sediment ($\mu gPbg^{-1}$)
720±5	430±20	290±14	10 800±500
800±6	500±30	316±16	11 400±600

Run III		
Initial Lead Concentration (μgmL^{-1})	Lead Concentration at Equilibrium (μgmL^{-1})	Lead Sorbed onto Sediment ($\mu gPbg^{-1}$)
20±2	0.4±0.7	480±50
40±2	1.9±0.2	890±60
60±2	4.4±0.5	1390±70
80±3	7.1±0.7	1870±80
120±3	12.5±1.5	2790±100
160±3	6.4±0.6	4060±90
200±3	33±2	4180±130

Table 4.13 cont.

Run III

Initial Lead Concentration (μgmL^{-1})	Lead Concentration at Equilibrium (μgmL^{-1})	Lead Sorbed onto Sediment ($\mu gPbg^{-1}$)
240±3	43±3	4870±160
280±4	59±2	5430±140
320±4	71±4	6240±200
360±4	91±4	6730±200
400±5	88±4	8350±200

Notes: (1) Total volume in experiment was 25 mL.

(2) Quantity of sediment used was 1.00±0.02g.

(3) Experiment carried out at 25±0.5°C.

(4) Values are mean±error.

Figure 4.11

Graph Showing Lead Sorption onto Sediment Sample (RC) from the Heathcote River.

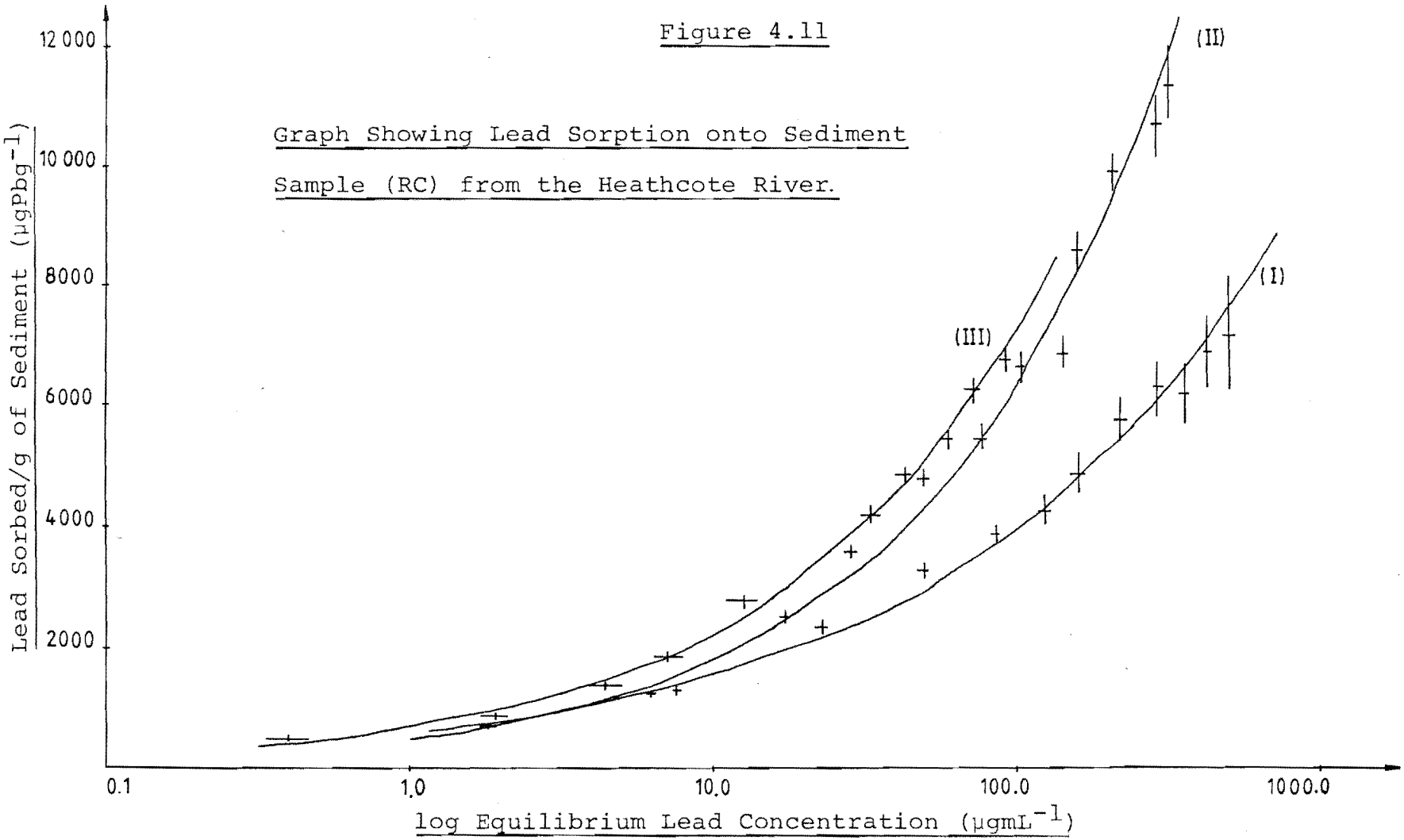
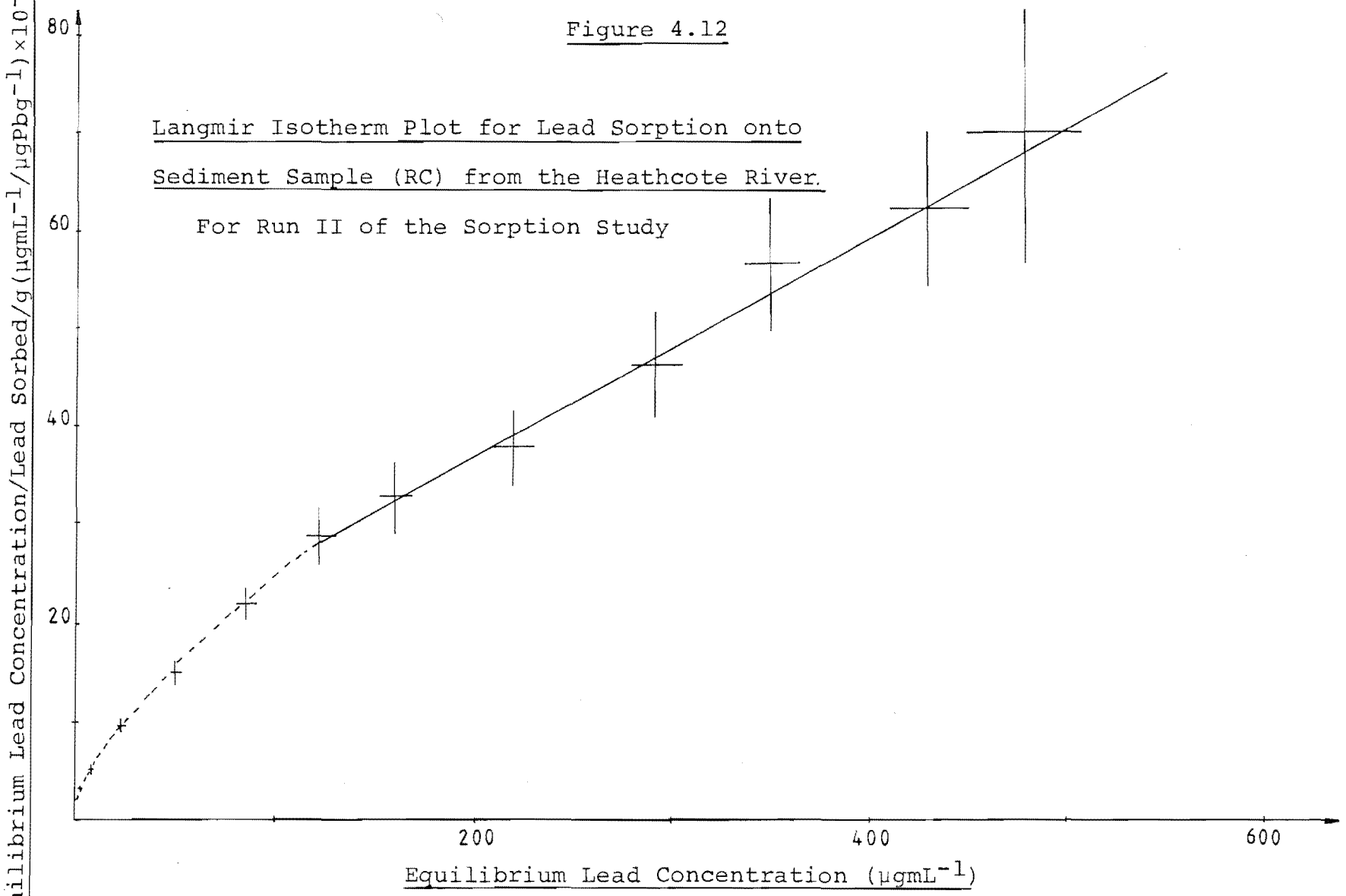


Figure 4.12

Langmir Isotherm Plot for Lead Sorption onto Sediment Sample (RC) from the Heathcote River. For Run II of the Sorption Study



clay and percentage of organic matter, the sediment sample labelled (RC) was separated into sand, silt, and clay fractions, and the organic matter content of each fraction determined. As well, the clay mineralogy of the clay fraction was determined by X-ray powder diffraction. The results of the separation of (RC) into sand, silt and clay fractions are given in Table 4.14. Also given in Table 4.14 are the results of a multielement analysis of the fractions obtained from the sediment sample (RC). From these results it is apparent that all elements are at higher concentration in the clay fraction. However, whether the higher level of these elements is due to the fact that they are bound to the clay minerals, the organics within the clay fraction, or to iron-manganese hydrous oxides, is not clear.

The clay fraction from (RC) as well as three clay fractions obtained from samples from profile I (see Figure 4.4) were analysed by powder X-ray diffraction to determine which clay minerals were present. For (RC) and the two upper samples from profile I the results were 85% illite, 10% kaolinite and 5% chlorite. In the deep sample from profile I the make up was the same except there was also approximately 5% paragonite and only 80% illite.

Because illite was the major component of the clay fraction and because lead is predominantly held in the clay fraction, a sorption experiment using identical conditions to those employed with sample (RC) but with pure illite was performed. (The results of this study are presented in Table 4.15 and Figure 4.13). From the data in Table 4.13 and 4.15 it was found that for a given

Table 4.14

Physical and Chemical Properties of River Sediemnt (RC) used in Sorption Studies.

	Sand	Silt	Clay
Percentage of Total	84±1	11±1	5±1
Percentage of Organic Matter	1.4±0.1	7.2±0.1	15.1±0.1
Cadmium Concentration	0.17±0.02	0.59±0.02	0.32±0.01
Copper Concentration	30±2	121±4	253±6
Chromium Concentration	19±1	50±2	150±9
Iron Concentration	9800±200	26 800±500	59 000±1700
Manganese Concentration	157±1	358±3	529±12
Lead Concentration	40±4	110±4	152±8
Antimony Concentration	<2	<2	<2
Zinc Concentration	8±0.5	29±1	59±1

Notes: (1) All element concentrations are in $\mu g g^{-1}$ on an ash weight (or organic free) basis.

(2) Values are mean±error.

Table 4.15

Sorption of Lead onto Illite.

Initial Lead Concentration (μgmL^{-1})	Lead Concentration at Equilibrium (μgmL^{-1})	Lead Sorbed onto Illite ($\mu gPbg^{-1}$)
30±0.5	11.8±0.4	920±50
60±2	37.5±1.0	1450±150
120±3	71±3	2390±270
180±3	131±2	2550±260
240±3	166±4	2750±350
300±3	219±5	4200±400
360±4	219±10	7100±700
460±4	325±10	6600±700
520±4	379±10	6900±700
600±5	463±10	6700±700
720±5	555±10	7900±700
800±6	637±16	8700±1200

Table 4.15 cont.

Notes: (1) Total volume in experiment was 25 mL.

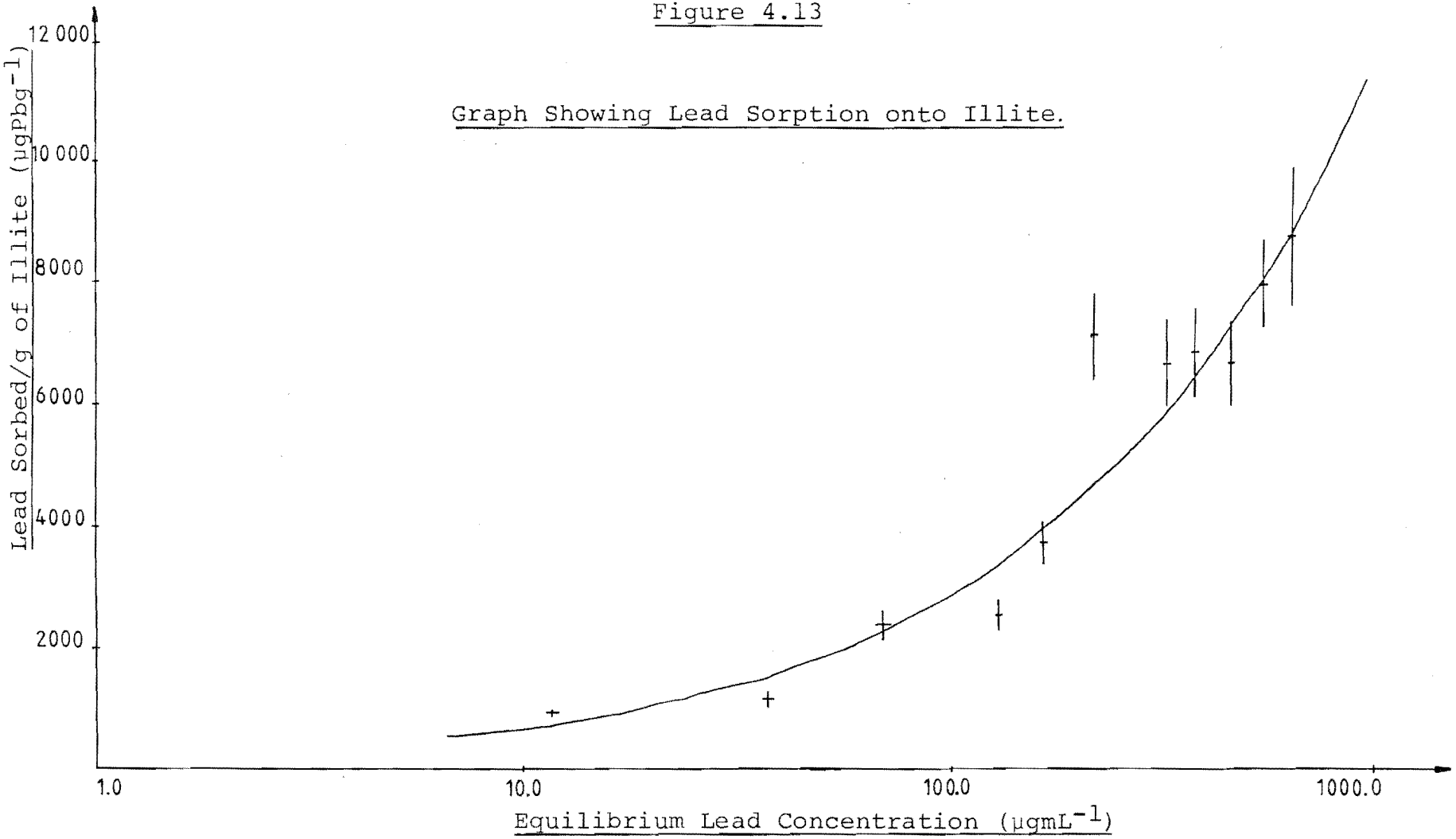
(2) Quantity of sediment used was 0.5±0.02 g.

(3) Experiment carried out at 25.0±0.5°C.

(4) Values are mean±error.

Figure 4.13

Graph Showing Lead Sorption onto Illite.



equilibrium solution concentration of lead, the quantity of lead sorbed per gram of sediment sample (RC) was twice that of illite under the same conditions. As illite however, makes up only 5% of the total weight of sediment sample (RC), then under the conditions of this experiment some other component of the sediment sample (RC) must be responsible for the bulk of the sorption. Scrdato and Estes (33) found that the sorption of lead onto illite was very pH dependent and they report sorption did not start to occur until pH 6. Since the natural pH of the sediment is greater than the pH used in the surface sorption experiments, it could be expected that more lead is sorbed onto the illite in the river than in the above experiment.

One possible explanation for the extra sorbing power of the sediment sample is the high iron concentration, 5.9% in the clay and 1.4% averaged over the sediment. It is well known that hydrous ferric oxides sorb strongly many metal ions including lead (34). Some evidence for this is the relatively high concentration of lead associated with the magnetic material in the sediments (see Table 4.7). However, organic matter can not be excluded as a likely candidate, as it makes up about 2.7% of the sediment, and has been found to have significant effect on lead sorption in river muds (32).

4.3.6 Multielement Analysis of a Profile from an Area of High Pollution on the Heathcote River.

To see how other elements were distributed with respect to depth and particle size within the polluted

areas of the Heathcote River, profile I (see Figure 4.14 and Section 4.3.2) was separated into clay, silt and sand fraction and each fraction was analysed with respect to pH, percentage of each particle size, percentage of organic material, cadmium, copper, chromium, iron, manganese, lead, antimony and zinc. The results of this investigation are given in Table 4.16 and Figures 4.14-4.24.

The analyses of the different elements show the expected trends. Lead and antimony have similar shaped curves, they both are used in the manufacture of lead-acid batteries and have similar aqueous chemistry. Cadmium and zinc both have similar chemistry and they both show the same pattern of distribution within the sediment. Also the increase in concentration down the profile is probably because, of all the metals studied, these are the most likely to be mobile as M^{2+} cations. Chromium, since it is used in the tanning factories upstream, is also higher in surface sediments than in lower sediments. Copper, iron and manganese are relatively constant in their presence at differing depths within the column, this is probably to be expected as their levels are typical of material not too highly polluted by the three metals.

One problem became evident while undertaking these analyses. Prior to the separation of the material into sand, silt, and clay, the lead concentration in these sediment samples had been determined, but when the total lead concentration was calculated for the sample for the sand, silt and clay fractions, the lead concentration was significantly less in all, except for the deepest fraction (I) 19-24 (see Table 4.17).

Table 4.16

Physical and Multielement Analysis of Profile (I) from the Heathcote River.

Depth (cm)	pH[1]	Particle Size[2]	% Total	Organics (%)	Cd	Cr	Cu	Fe	Mn	Pb	Sb	Zn
0-2	5.4	Sand	49.5	3.1	0.26	56	31	16 400	83	650	31	17
		Silt	38.8	7.3	0.63	195	82	36 300	128	1075	44	37
		Clay	11.7	15.6	0.60	530	220	67 500	143	3050	130	79
10-14	5.9	Sand	48.6	7.0	0.25	98	43	23 100	79	245	8	20
		Silt	35.9	11.5	0.81	540	173	37 600	97	745	20	45
		Clay	15.5	19.7	0.79	1210	320	59 100	109	930	42	68
19-24	5.7	Sand	52.0	16.7	0.88	114	29	25 200	90	116	10	51
		Silt	40.8	11.9	1.54	390	71	36 200	138	214	17	89
		Clay	7.2	17.0	1.57	1020	185	66 400	136	483	14	142

Notes: (1) pH taken in distilled water (see Table 4.6).

(2) Sand=particles>20μm. Silt=particles<20μm and>2μm. Clay=particles<2μm.

(3) All elements have concentration in $\mu g g^{-1}$ on ash weight basis.

Table 4.16 cont.

Notes: (4) Error on determination is less than 5% except for antimony where error is less than 10%.

(5) Depth is in cm below the surface.

(6) Profile (I) as described in Figure 4.4.

(7) "Organics" was determined by weight lost at 450°C after 16 hours.

Figure 4.14

Graph Showing Change in pH with Depth Below Surface for Sediment Profile I.

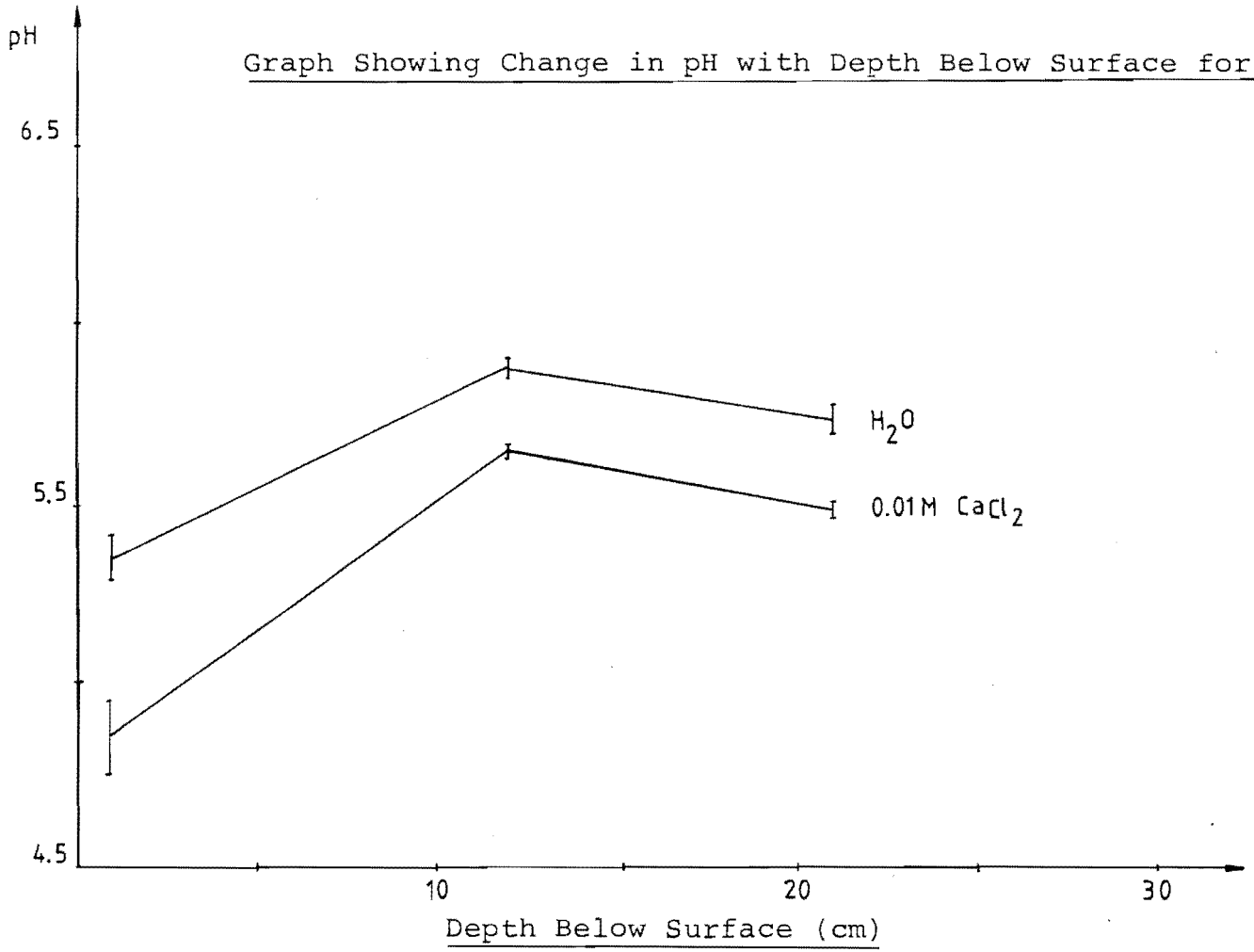


Figure 4.15

Graph Showing Percentage Composition of Profile I with Respect to Sand (<20μm), Silt (2-20μm) and Clay (>20μm).

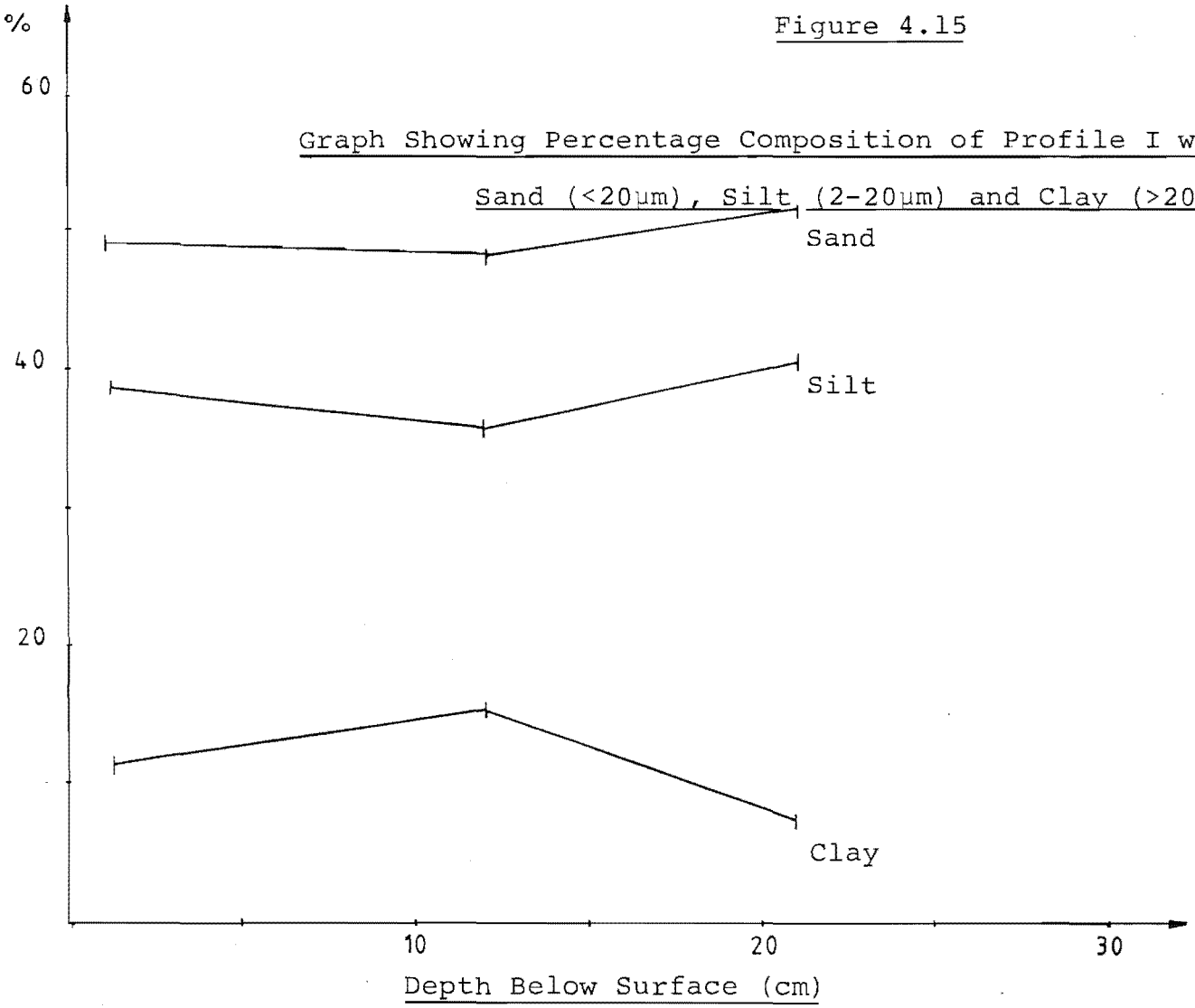


Figure 4.16

Graph Showing the Percentage of "Organic" Matter in Profile I with Respect to Sand, Silt and Clay as well as Depth Below Surface.

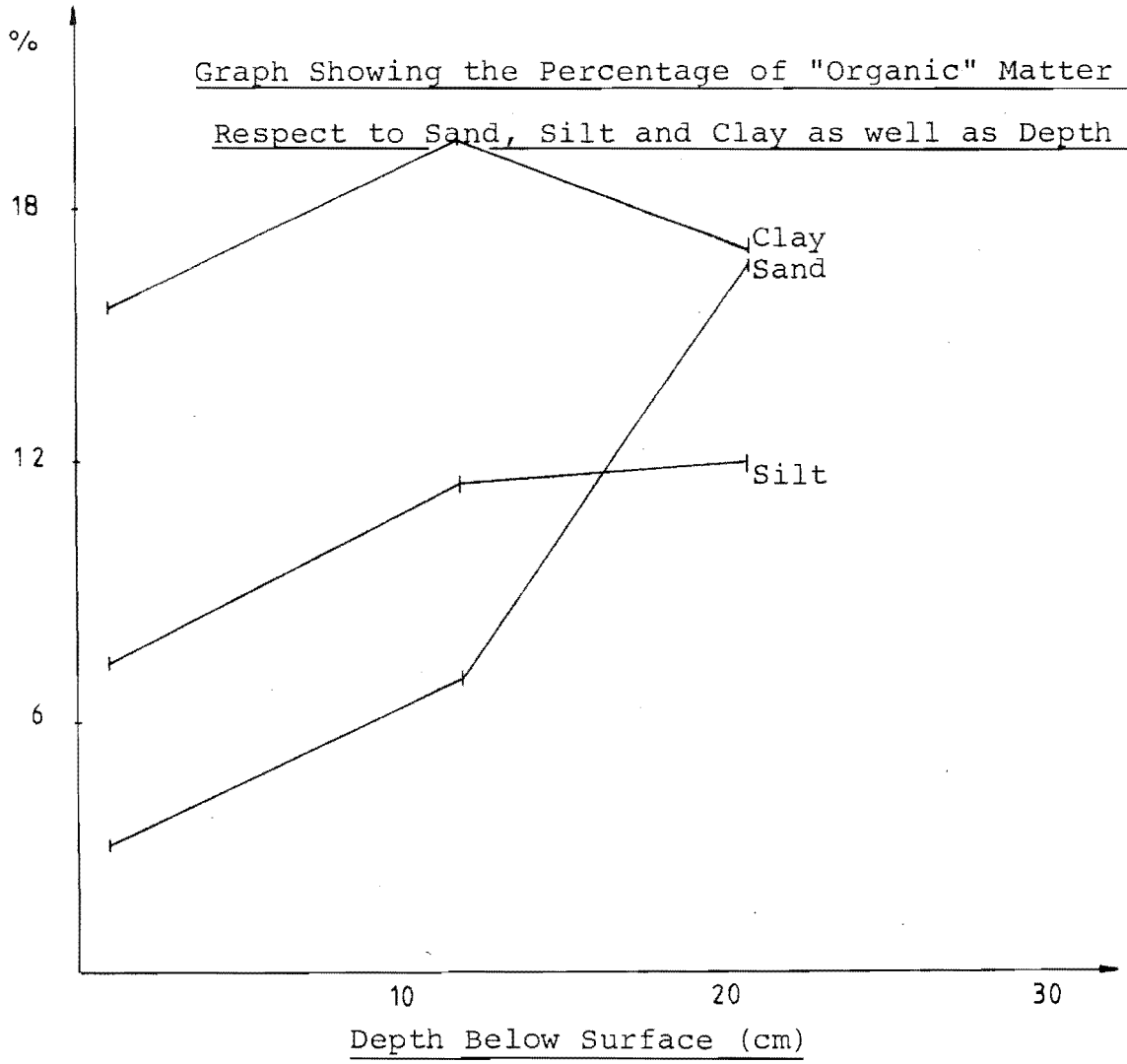
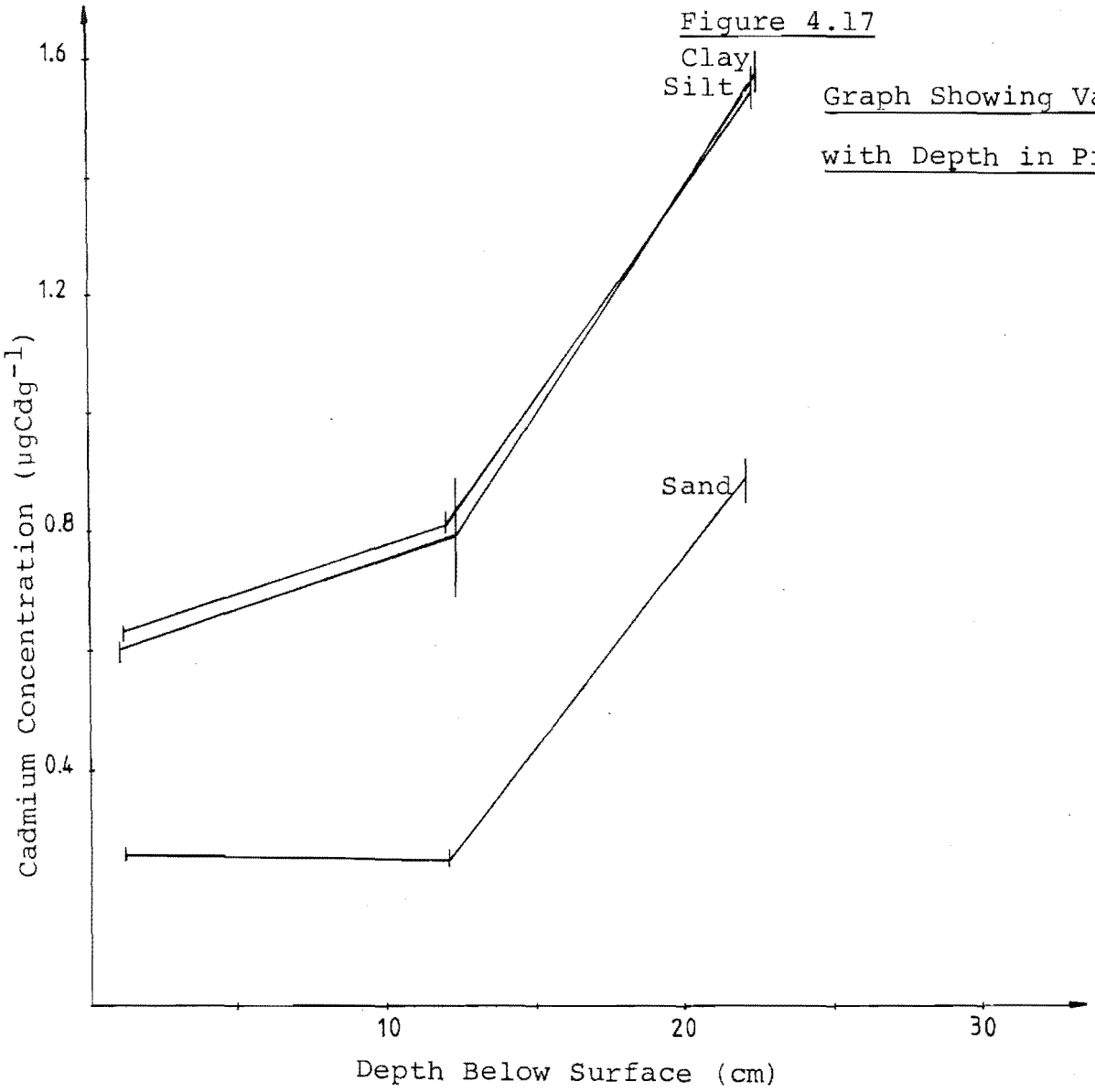


Figure 4.17



Graph Showing Variation in Cadmium Concentration with Depth in Profile I and with Respect to Sand, Silt and Clay.

Figure 4.18

Graph Showing Variation in Chromium Concentration with Depth in Profile I and with Respect to Sand, Silt and Clay Fractions.

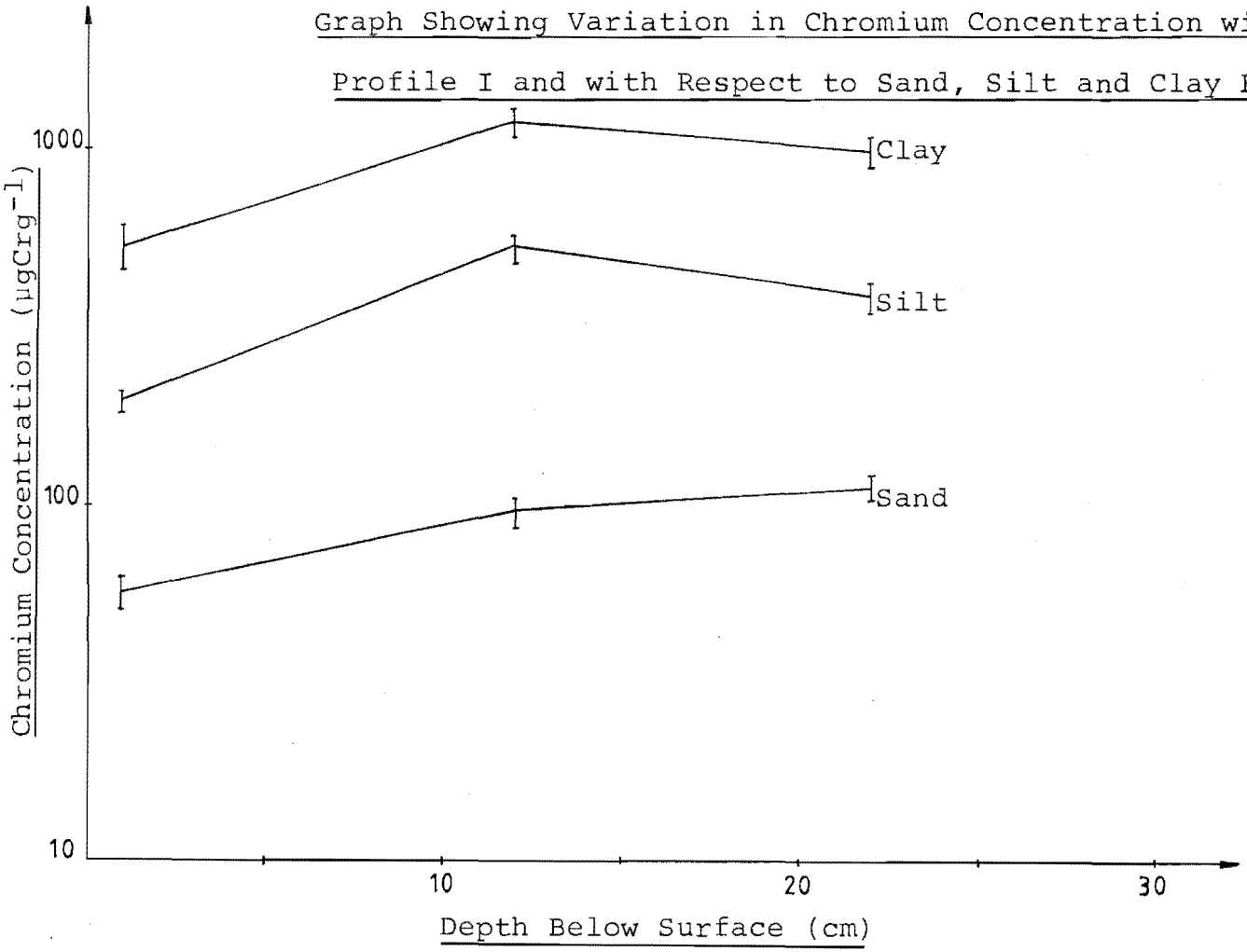


Figure 4.19

Graph Showing Variation in Copper Concentration with Depth in Profile I and with Respect to Sand, Silt and Clay Fractions.

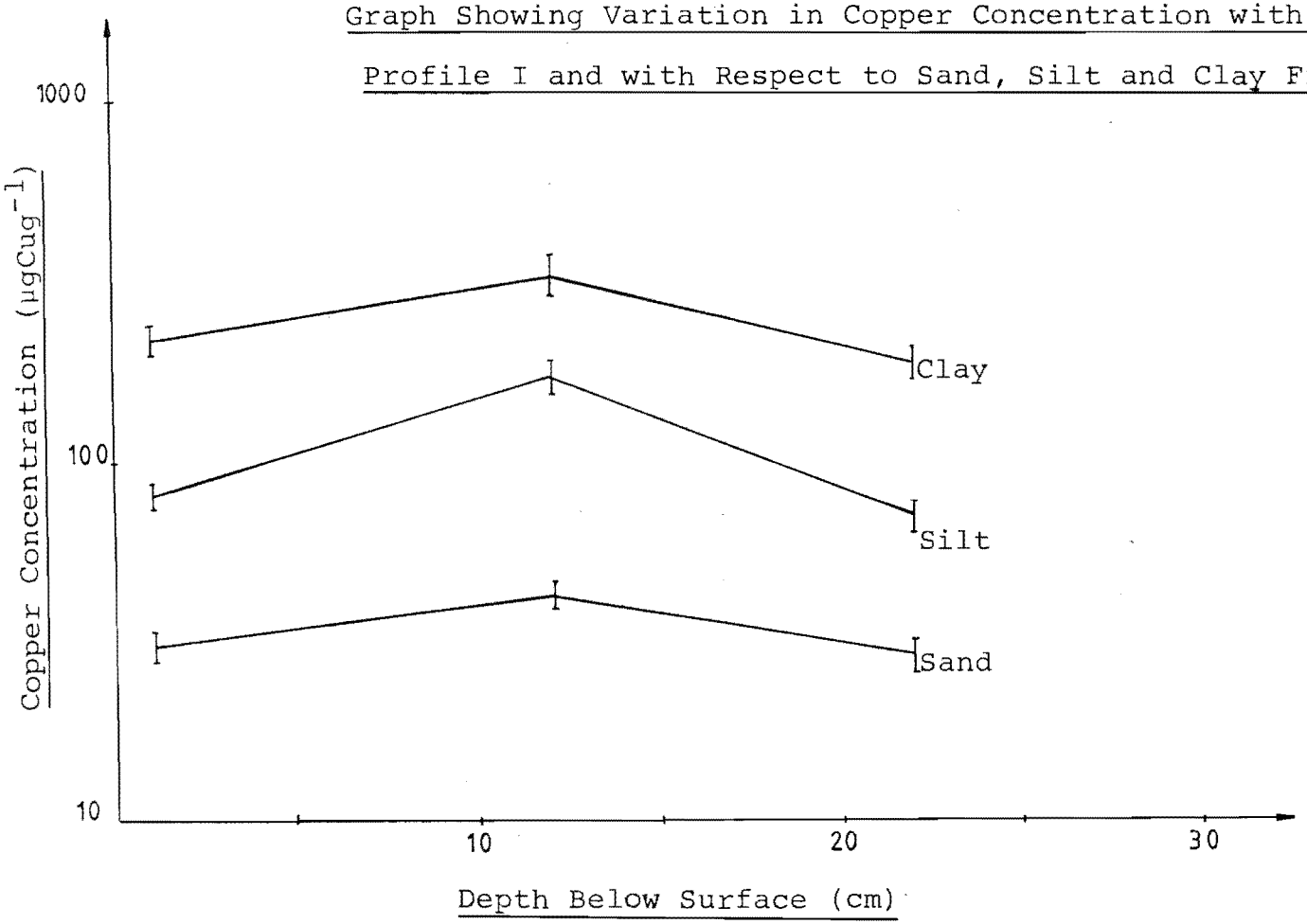


Figure 4.20

Graph Showing Variation in Iron Concentration with Depth in Profile I and with Respect to Sand, Silt and Clay Fractions.

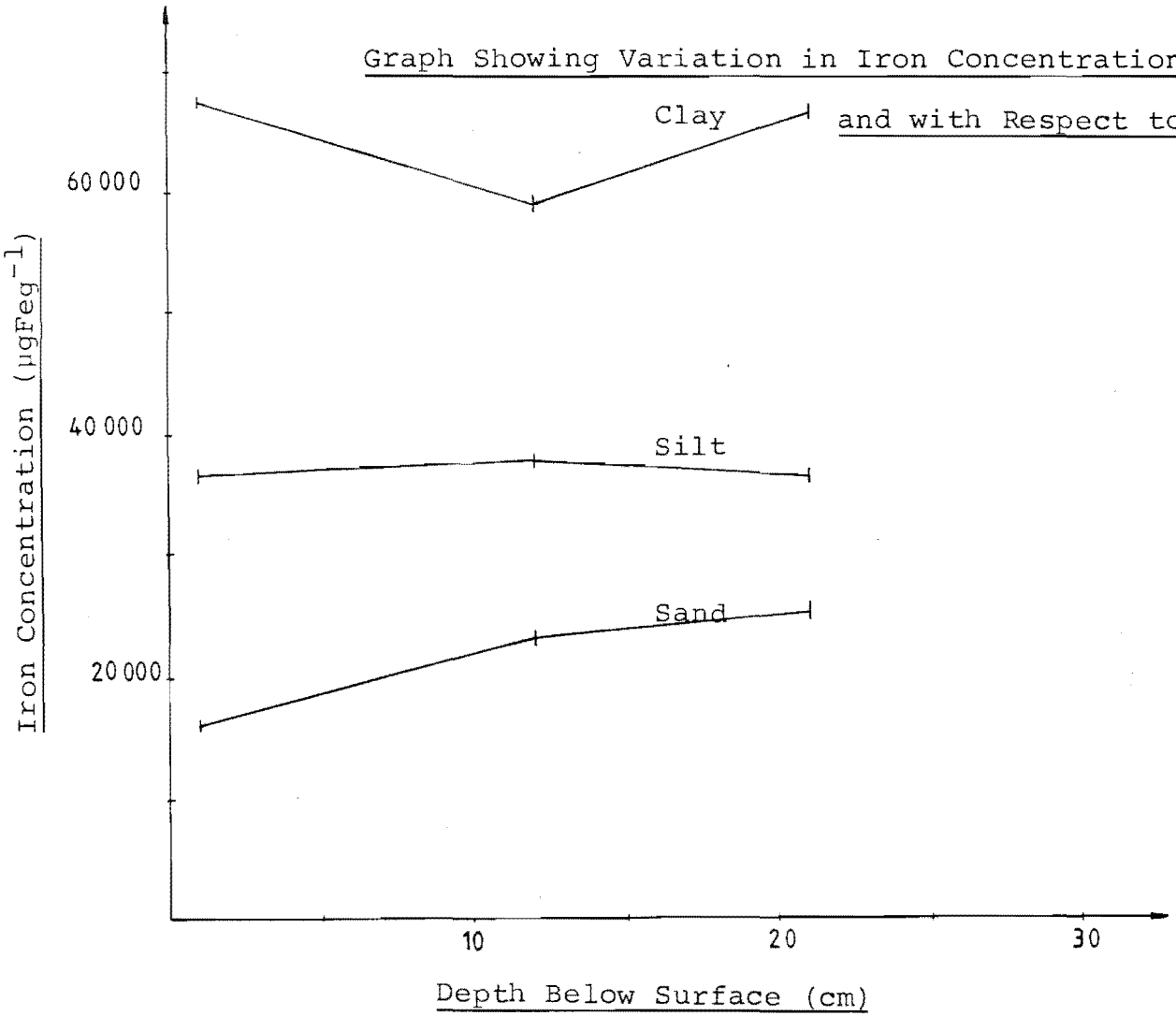


Figure 4.21

Graph Showing Variation in Manganese Concentration with Depth in Profile I and with Respect to Sand, Silt and Clay Fractions.

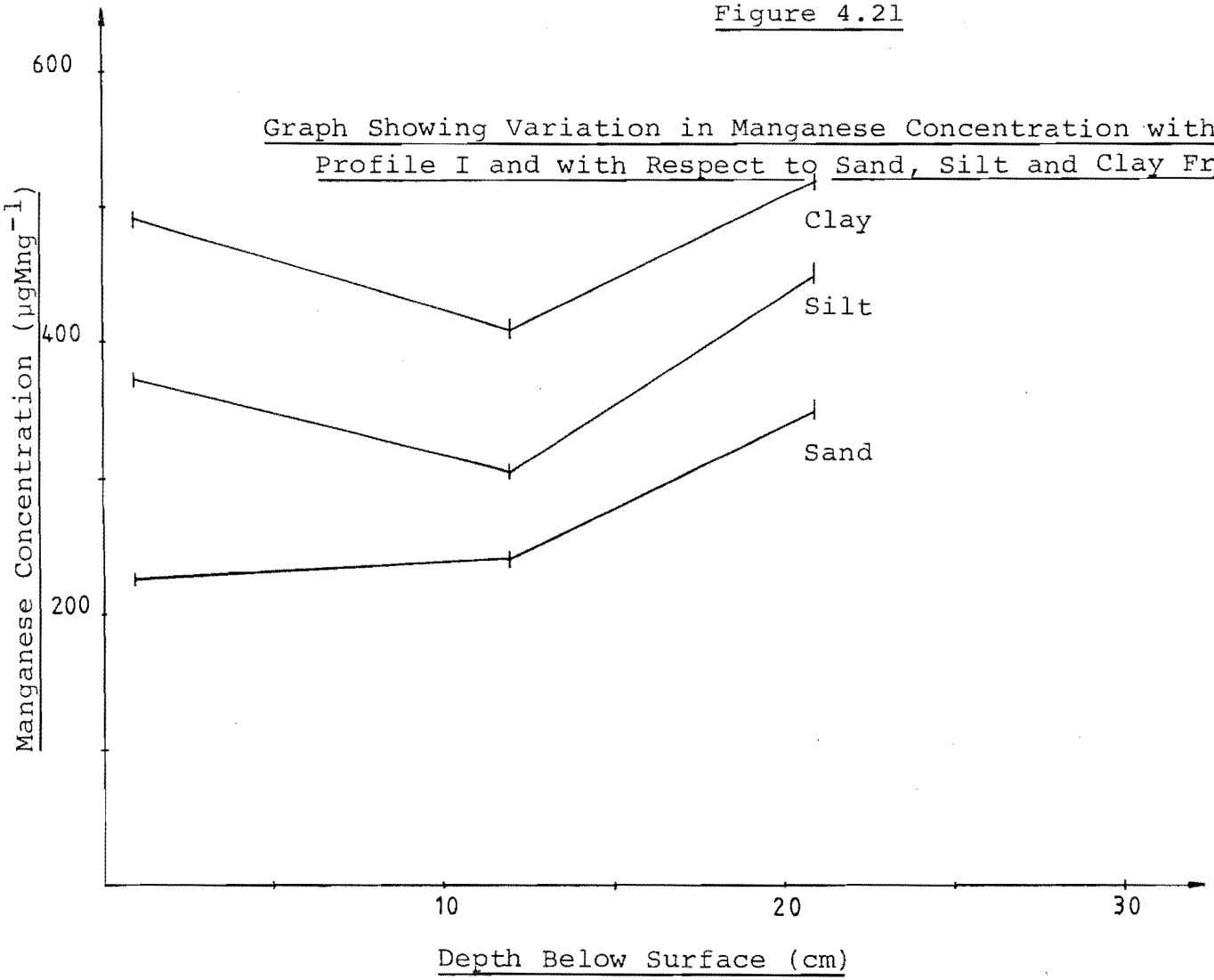


Figure 4.22

Graph Showing Variation in Lead Concentration with Depth in Profile I and with Respect to Sand, Silt and Clay Fractions.

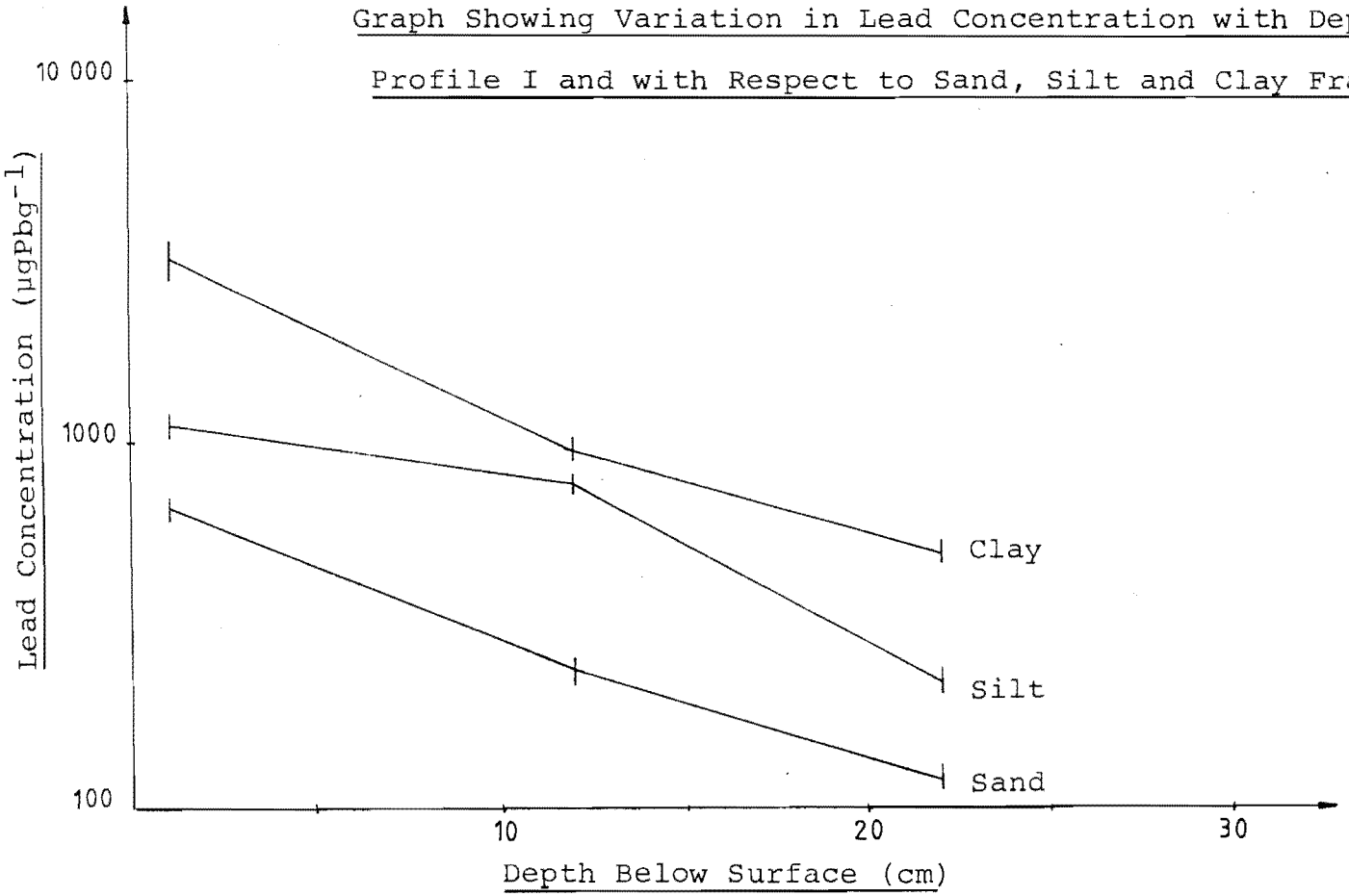


Figure 4.23

Graph Showing Variation in Antimony Concentration with Depth in Profile I and with Respect to Sand, Silt and Clay Fractions.

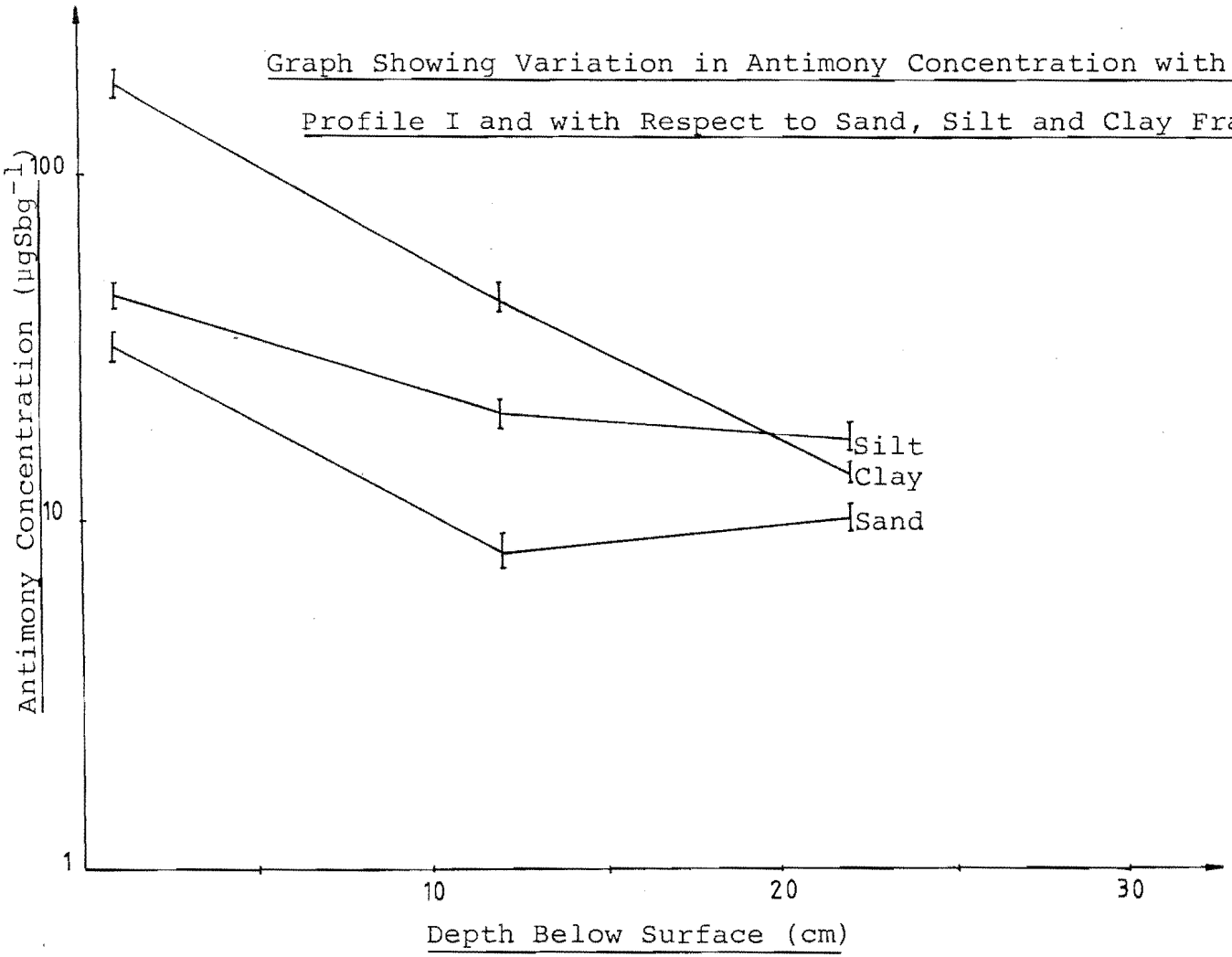


Figure 4.24

Graph Showing Variation in Zinc Concentration with Depth in Profile I and with Respect to Sand, Silt and Clay Fractions.

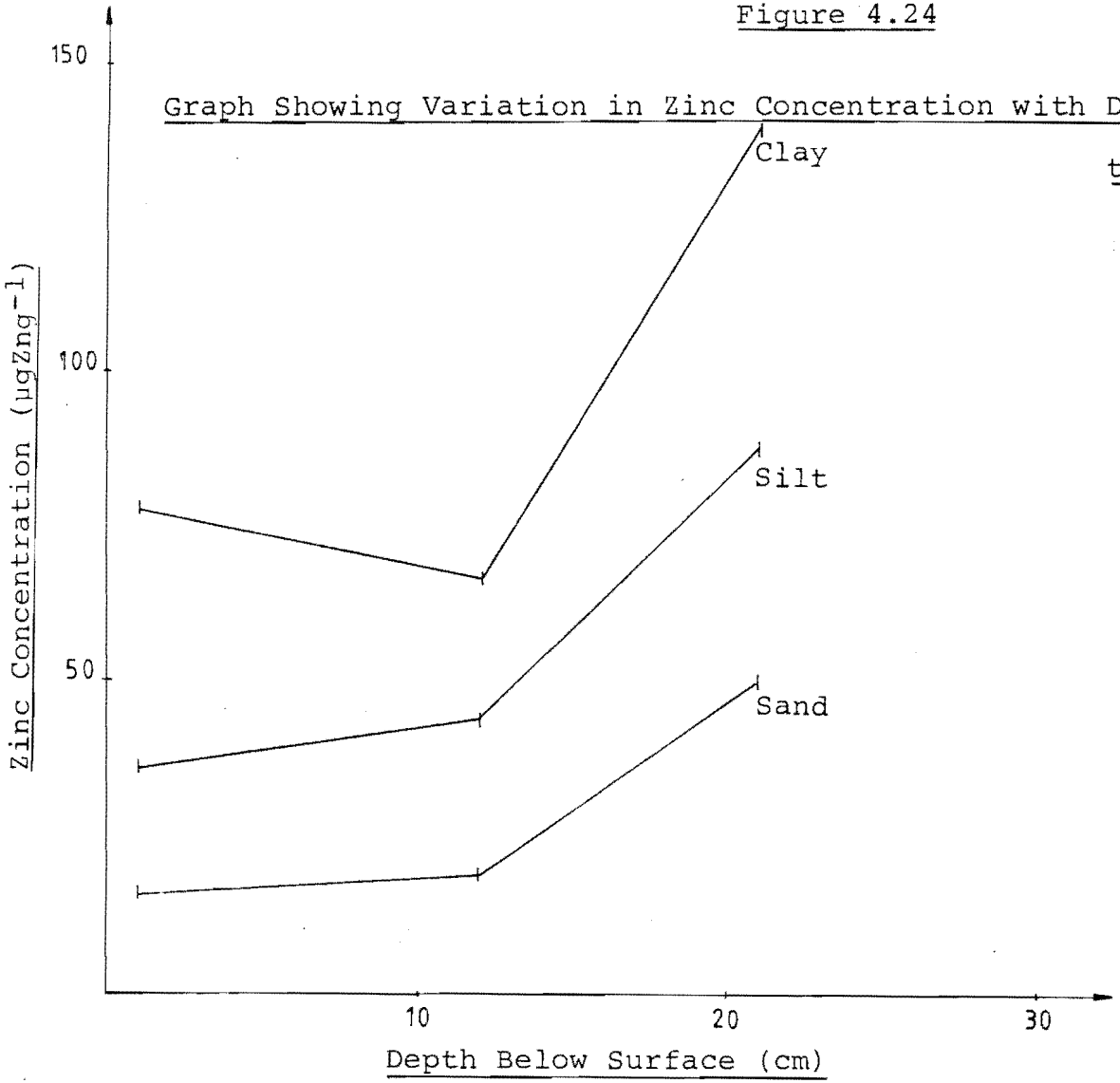


Table 4.17

Lead Concentration in Sediment Samples Before and After Separation into Sand, Silt and Clay Fractions

Lead Concentration in $\mu gPbg^{-1}$ (dry weight).

Sample	Lead Concentration Prior to Separation into Fraction (1)	Lead Concentration After Separation into Fractions (2)
RC	87±5	51±5
(I) 0-2	3100±100	1000±100
(I) 10-14	1600±40	460±50
(I) 19-24	130±10	150±10

Notes:

(1) HNO_3 digestion (95-100% extraction of lead).

(2) $HF/HClO_4$ digestion (100% extraction of lead).

The most likely reason for the difference is that lead was leached out of the samples while carrying out the particle size separation. Whether this occurred for other elements is not known, but as cadmium and zinc are generally more soluble than lead, it would be expected these elements could have been severely affected. Tan (2) found the concentration of cadmium was in the range 0.33-0.93 $\mu gCdg^{-1}$ near the site where the profile was obtained. The value for the surface concentration of cadmium

obtained from this study was 0.48 $\mu gCdg^{-1}$ which is at the low end of Tan's (2) range. Because of the low solubility of iron and manganese there is probably little loss of these elements. A point of interest which supports the suggestion that lead has been washed out is that for sample (I) 19-24, there is no apparent loss of lead and this may be due to the fact that the much less soluble lead sulphide is a dominant form at the lowest depths in the profile.

When the levels of these elements are compared with other rivers elsewhere in the world, it would appear that cadmium and zinc levels are low, as levels of cadmium range from 3-88μgg^{-1} with zinc ranging from 100-3100μgg^{-1}, in the Rhine (35). The Heathcote is not as polluted as the Derwent Estuary where levels of up to 1400$\mu gCdg^{-1}$ were found near Hobart and zinc levels in the sediment were as high as 10% (19). The levels in the Heathcote River appear to be comparable with those of Poole Harbour, Dorset, where cadmium levels range from less than 1 to 10$\mu gCdg^{-1}$ and zinc concentrations range from 3-217$\mu gZng^{-1}$.

Copper and chromium levels in the Heathcote River appear to be similar to other rivers where industrial waste is discharged. The sediments in the River Elbe contain up to 1250$\mu gCug^{-1}$ and 770$\mu gCrg^{-1}$ (16) in the River Rhine levels of copper range from 42-408$\mu gCug^{-1}$ and chromium levels range from 33-1200$\mu gCrg^{-1}$. Sediment levels from near the Los Angeles County outfall system show chromium levels up to 1400$\mu gCrg^{-1}$ and copper levels of up to 940$\mu gCug^{-1}$, the discharge from this outfall being both of an industrial and municipal waste (36).

From these comparisons it would appear that the lower

reaches of the Heathcote are either somewhat polluted or strongly polluted, depending on which metals are being considered.

4.3.7 Temporal Variation in Lead Concentration in the Heathcote River Sediments.

It was found (Chapter 2) that lead levels in cockles decreased over time period of 1979-1982 and as the lead level in New Zealand petrol had not decreased and the number of cars had not diminished then it was believed that the major source of lead to the shellfish must be of an industrial source. The most likely industrial source was the battery factory on the banks of the Heathcote River. To investigate whether the quantity of lead being released by the battery factory had also changed over the same time period, three sediment profiles were taken from near the battery factory outfall pipe in the Heathcote River. The results of the analysis of these three profiles are given in Table 4.18 and Figure 4.25. The results show a decrease in the lead concentration of the surface sediments especially from the end of 1979 to mid 1982 and hence support the notion that the major source of lead for the cockles in the Avon-Heathcote Estuary is lead waste output from the battery factory. The difference is unlikely to be experimental error as it may be seen from the data that very similar levels of lead are obtained for the two profiles collected only two months apart.

Table 4.18

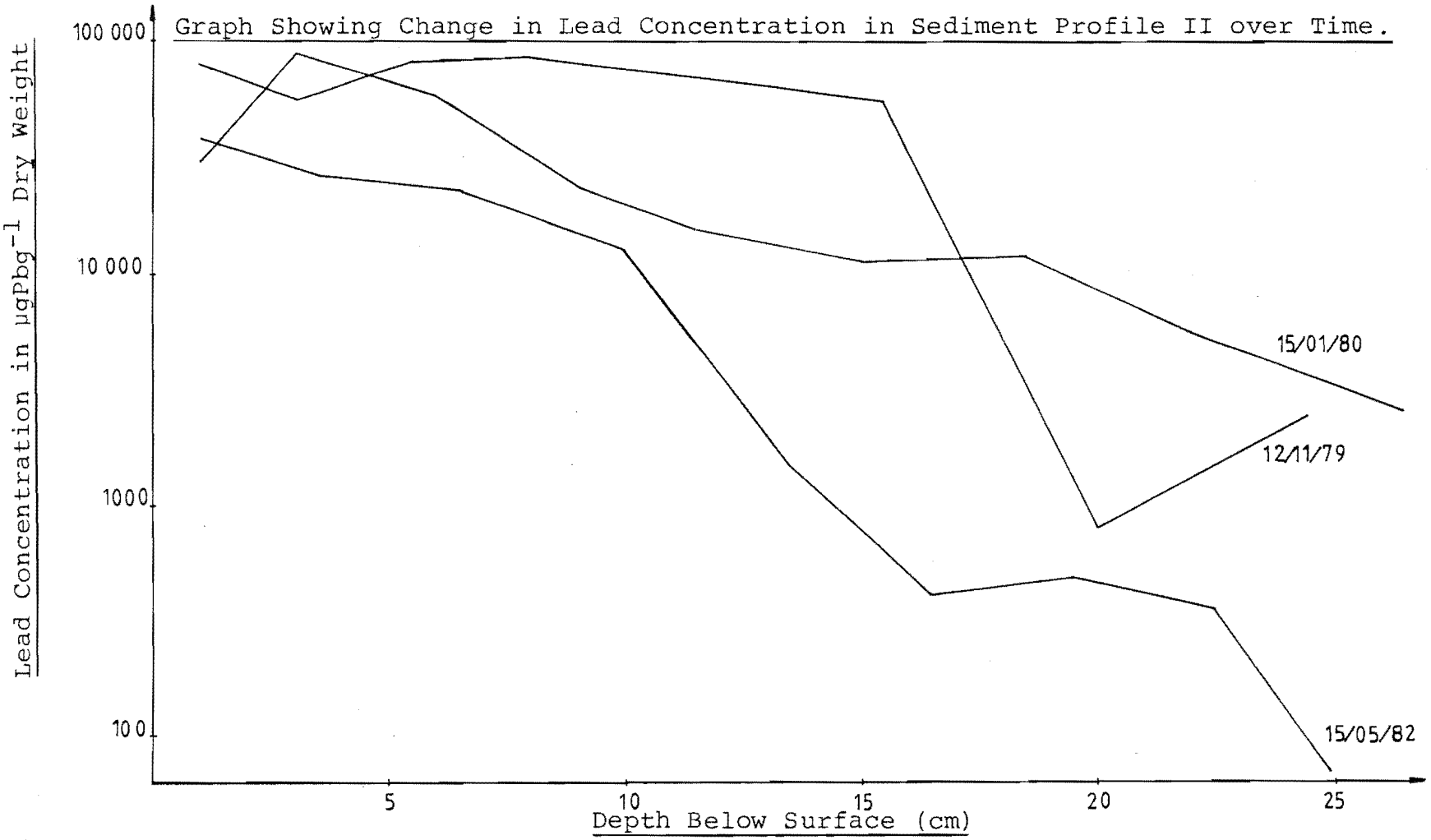
Variation in Lead Concentration with Time in Profile II from the Heathcote River.

Time	12/11/79		15/01/80		15/05/82	
	Depth	Concentration	Depth	Concentration	Depth	Concentration
	0-2	80 000±2000	0-2	31 500±1300	0-2	39 500±400
	2-4	58 000±2000	2-4	89 300±4500	2-5	27 400±400
4-7	4-7	82 000±3000	4-8	61 000±4000	5-8	23 600±300
	7-9	87 000±3000	8-10	23 700±1100	8-12	12 800±300
	9-13	76 000±3000	10-13	15 900±800	12-15	1500±20
13-18	13-18	34 000±2000	13-17	11 500±900	15-18	420±20
	18-22	800±40	17-20	12 300±900	18-21	800±20
	22-27	2300±100	20-24	5500±700	21-24	370±20
			24-25	3500±100		
			25-28	2500±100		

Notes: (1) Depth in cm below the surface. (2) Date in the form of day/month/year.

(3) Lead Concentration in $\mu gPbg^{-1}$ dry weight. (4) Values are mean±error

Figure 4.25


Graph Showing Change in Lead Concentration in Sediment Profile II over Time.
Lead Concentration in μgPbg⁻¹ Dry Weight
100 000
10 000
1000
100
5
10
15
20
25
Depth Below Surface (cm)
15/01/80
12/11/79
15/05/82

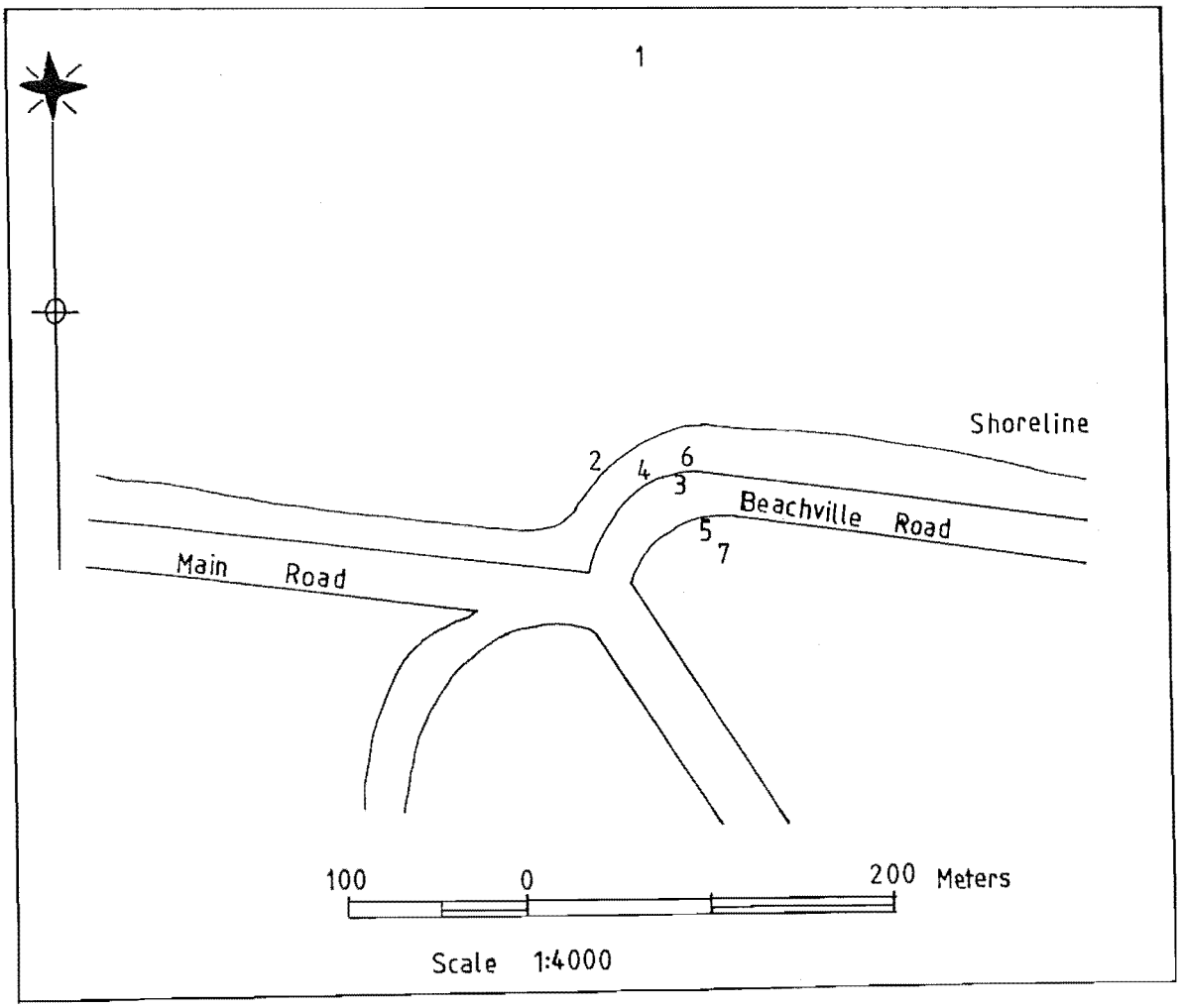
4.3.8 Checks on the Immediate Environmental Concentrations of Lead near the Shellfish Sampling Site.

In order to check that the lead concentrations, as seen in the cockles from the Avon-Heathcote Estuary, are representative of levels of lead in the water (and presumably originating from the lead source in the Heathcote River) and are not influenced by local sources of lead in the immediate environment of the sampling site, analysis of environmental samples from near the sampling site was undertaken. Two types of samples were obtained, the first being soil and sediment samples around the site and the second being vegetation samples. The collection sites and type of material are given in Figure 4.26 and the results of the analyses are given in Table 4.19. From these results it would appear that there is no major local source of lead in the close vicinity of the sampling site.

The higher levels of lead found on the land side of the road, are probably due to the fact that the predominant wind in the area would blow lead emitted by cars away from the estuary side of the road. The rapid fall off in lead concentrations from the road to the estuary also indicates that adjacent local lead sources do not appear to influence the concentrations of lead near the shellfish and hence the shellfish would appear to reflect lead levels within the Avon-Heathcote Estuary rather than the lead levels in the local environmental vicinity. As demonstrated, the source of the lead is most likely the battery factory.

Figure 4.26

Environmental Sampling Sites Near Shellfish Sampling Site.



Site Description

(1) Shellfish Sampling Site.

(2) Sediment Sample taken near the road.

(3) Road dust from Beachville Rd.

(4) Soil from the estuary side of the road.

(5) Soil from the land side of the road.

(6) A sample of grass from the estuary side of the road.

(7) A sample of tree leaves from the land side of the road.

Table 4.19

Analysis for Lead in Environmental Samples near Shellfish Sampling Site in the Avon-Heathcote Estuary.

Section A: Sediment, Soils and Road Dust.

Site No.	Description of Sample	Lead Concentration ($\mu gPbg^{-1}$)
1	Sampling site, estuary sediment	10.5 ±0.4
2	Roadside estuary sediment	34.2 ±4.0
3	Road dust	510 ±70
4	Soil, estuary side of road	120 ±25
5	Soil, land side of road	127 ±5

Section B: Vegetation.

Site No.	Description of Sample	Lead Concentration ($\mu gPbg^{-1}$)
1	Sea lettuce from sampling site	1.7±0.2
6	Grass from roadside	18.6±1.3
7	Tree leaves, land side of grass	9.2±0.9

Table 4.19 cont.

Notes: (1) For section A and B, lead concentration is in $\mu gPbg^{-1}$ dry weight.

(2) Values are mean±error.

4.3.9 The Presence of Organolead Compounds in Shellfish.

Because of suggestions that lead can be bio-methylated by organisms living in the sediment but not identified (3-6) and the evidence of tetra-alkyl lead compounds being produced by estuarine sediments (7), it was decided to see if organo-lead compounds were accumulated by cockles. However, no organo-lead compounds could be found in either the benzene or ethyl acetate extracts as both contained insufficient lead to give a signal greater than the noise levels for the method of analysis employed. This would place an upper limit of 5% on the percentage of organic lead in cockes obtained from the Avon-Heathcote Estuary.

This negative result could be used to support the notion that bio-methylation does not occur in sediments for lead (8, 9) but it is more likely due to the low levels of lead pollution in the actual Avon-Heathcote Estuary. A more sensitive method would be necessary to detect the presence of bio-methylated lead compounds.

4.4.1 Conclusion.

A number of results and deductions from these results, have been given in this chapter, a few of the more significant conclusions are gathered here.

(1) Because of the higher industrial build-up along the banks of the Heathcote River, the Heathcote River is considerably more polluted than the Avon River.

(2) Because of the geological nature of the Avon-Heathcote

Estuary there is no evidence for the accumulation of trace metals within this estuary, except for some accumulation at Pleasant Point.

(3) The Heathcote River in its lower reaches is highly polluted by lead, antimony and chromium.

(4) Lead levels in sediments of the rivers are due to industrial discharge and road dust wash off from storm water discharges.

(5) The chemistry of lead within the sediment is dependent upon the pH, and the reductive potential within the sediment, and lead compounds change from $PbCO_3$ to $PbSO_4$ to PbS with increasing depth below the surface of sediment.

(6) Lead and other trace elements are found predominantly in the clay fraction of the sediment and in areas of sediment with high organic content and magnetic (ferro-manganese) material.

(7) There appears to be no evidence to support the possible bio-methylation of lead to organo-lead compounds.

References.

1. Macpherson, J.M. Response to urbanization of the Avon-Heathcote Estuary, Christchurch, New Zealand. Environ. Geol., 3, 23-27, (1979).

2. Tan Seow Yong. Lead in the Christchurch environment. M.Sc. Thesis, Univ. of Canterbury, 1979, pp 161.

3. Wong, P.T.S; Chau, Y.K; Luxon, P.L. Methylation of lead in the environment. Nature, 253, 263-264, (1975).

4. Schmidt, U; Huber, F. Methylation of organolead and lead (II) compounds to $(CH_3)_4Pb$ by microorganisms. Nature, 259, 157-158, (1976).

5. Dumas, J.-P; Pazdernik, LeR; Belloncik, S; Bouchard, D; Vaillancourt, G. Methylation du plomb en milieu aquatique. Proc. 12th Canadian Symp. 1977: Water Pollution Research Canada, pp 91-100.

6. Thompson, J.A.J; Crerar, J.A. Methylation of lead in marine sediments. Mar. Pollut. Bull., 11, 251-253, (1980).

7. Harrison, R.M; Laxen, D.P.H. Natural source of tetraalkyllead in air. Nature, 275, 738-740, (1978).

8. Jarvie, A.W.P; Markall, R.N; Potter, H.R. Chemical alkylation of lead. Nature, 255, 217-218, (1975).

9. Reisinger, K; Stoeppler, M; Nürnberg, H.W. Evidence for the absence of biological methylation of lead in the environment. Nature, 291, 228-230, (1981).

10. Fergusson, J.E; Simmonds, P.R. Heavy metal pollution of an intersection involving a busy urban road in Christchurch, New Zealand. I. Levels of Cr, Mn, Fe, Ni, Cu, Zn, Cd and Pb in street dust. N.Z. J. Sci., 26, 219-228, (1983).

11. Linton, R.W; Natusch, D.F.S; Solomon, R.L; Evans, C.A. Jr. Physicochemical characterization of lead in urban dusts. A microanalytical approach to lead tracing. Environ. Sci. Technol., 14, 159-164, (1980).

12. Tanner, C.B; Jackson, M.L. Nomographs of sedimentation times for soil particles under gravity or centrifugal acceleration. Soil Sci. Soc. Am. Proc., 12, 60-65, (1947).

13. Powder diffraction file compiled and published by J.C.P.D.S., 1980.

14. Brindley, G.W; Brown, G. (Eds.) Mineralogical Society Monograph, No. 5: crystal structures of clay minerals and their X-ray identification. Mineralogical Society: London, Engl. 1980, pp 495.

15. Hayakawa, K. Microdetermination and dynamic aspects of in vivo alkyl lead compounds. Part I. Analytical methods. JPN. J. Hyg., 26, 377-385, (1971).

16. Müller, G; Förstner, U. Heavy metals in sediments of the Rhine and Elbe Estuaries: mobilisation or mixing effect? Environ. Geol., 1, 33-39, (1975).

17. Cooper, B.S; Harris, R.C. Heavy metals in organic phases of river and estuarine sediment. Mar. Pollut. Bull., 5, 24-26, (1974).

18. Harding, S.C; Brown, H.S. Distribution of selected trace elements in sediments of Pamlico River Estuary, North Carolina. Environ. Geol. (N.Y.), 1, 181-191, (1975).

19. Bloom, H; Ayling, G.M. Heavy metals in the Derwent Estuary, Environ. Geol., 2, 3-22, (1977).

20. Boydon, C.R. Distribution of some trace metals in

Poole Harbour, Dorset. Mar. Pollut. Bull., 6, 180-187, (1975).

21. Skei, J.M; Price, N.B; Calvert, S.E; Holtedahl, H. The distribution of heavy metals in sediments of Sörfjord, West Norway. Water, Air Soil Pollut., 1, 452-461, (1972).

22. Houba, C; Remacle, J. Distribution of heavy metals in sediments of the River Vesdre (Belgium). Environ. Technol. Lett., 3, 237-240, (1982).

23. Pasternak, Kazimierz. The accumulation of heavy metals in the bottom sediments of the River Biala Przemsza as an indicator of their spreading by water courses from the centre of zinc and lead mining and smelting industries. Acta. Hydrobiol., 16, 51-63, (1974).

24. Becker, R. Geochemical study of sediments of the Blies River, Saarland, Naturwissenschaften, 63, 144-145, (1976).

25. Warne, S. St. J; Bayliss, P. The differential thermal analysis of cerussite. Am. Mineralogist, 47, 1011-1023, (1962).

26. McLaughlin, R.J.W. Other minerals chpt. 14, the differential thermal investigation of clays. Edit. MacKenzie, R.C. Mineralogical Society, London, 1957.

27. Byrne, R.H. Inorganic lead complexation in natural seawater determined by U.V. spectroscopy. Nature, 290, 487-489, (1981).

28. Sipos, L; Valenta, P; Nuernberg, H.W; Branica, M. Voltammetric determination of the stability constants of the predominant labile lead complexes in seawater. Lead Mar. Environ. Proc. Int. Experts Discuss., 1977,

(Pub. 1980), 61-76. Edited by Branica, M; Konrad, Z; Pergamon: Oxford, Engl.

29. Jonasson, I.R. Detailed hydrogeochemistry of two small lakes in the Grenville Geological Province. Geol. Surv. Can., Pap., 1976, (76-13), pp 37.

30. Naruse, Y; Ando, R; Tsutiyama, F; Sugiyama, H. Behaviour of heavy metals in estuary. Zenkoku Kogaiken Kaishi, 5, 29-32, (1980).

31. Griffen, R.A. Au, A.K. Lead adsorption by montmorillonite using a competitive Langmuir equation. Soil Sci. Soc. Am. J., 41, 880-882, (1977).

32. Salim, R; Cooksey, B.G. Kinetics of the adsorption of lead onto river mud. Plant Soil, 54, 399-417, (1980).

33. Scrudato, R.J; Estes, E.L. Clay-lead sorption relations. Environ. Geol., 1, 167-170, (1975).

34. Swallow, K.C; Hume, D.N; Morel, F.M.M. Sorption of copper and lead by hydrous ferric oxide. Environ. Sci. Technol., 14, 1326-1331, (1980).

35. Banat, K; Förstner, U; Müller, G. Schwermetalle in sedimenten von Donau, Rhein, Ems, Weser und Elbe im Bereich der Bundesrepublik Deutschland. Naturewissenschaften, 59, 525-528, (1972).

36. Hershelman, G.P; Schafer, H.A; Jan, T-K; Young, D.R. Metals in marine sediments near a large California (U.S.A.) municipal outfall. Mar. Pollut. Bull., 12, 131-134, (1981).

Chapter 5

Lead Concentrations in Human Teeth - A Review

5.1.1 Introduction

The two principal objectives in the study of trace levels of lead in human teeth are, to investigate the effect of trace amounts on caries prevalence, and to use teeth as an indicator of lead exposure in the population. The present review probes past work on trace lead levels in human teeth with an emphasis on the applicability of the methods used for the trace analysis. Consideration will also be given to the various factors which affect the measured levels of lead found in a particular tooth or group of teeth.

The discovery that fluoride in teeth decreases the prevalence of caries led to investigations of the effects of lead and other trace metals on caries prevalence. While some authors conclude that lead actually increases the caries prevalence, other authors claim a decrease. However, both findings are barely significant. It would appear from the literature that lead has a minimal effect on caries prevalence. (1, 2, 3, 4, 5).

Lead has been shown to accumulate in heavy human tissue such as teeth and bones and is relatively immobile in teeth. Also since teeth are readily obtainable they have been widely used in monitoring surveys which are concerned with the levels of lead in humans. Part of the aim of this review is to investigate the advantages and the disadvantages of

using various parts of teeth in monitoring surveys. Factors affecting lead levels in teeth such as age of donor, geographical residence, type of tooth, section of tooth analysed, occupational exposure and sex as well as analytical techniques will also be discussed.

5.2 Analytical Methods

5.2.1 Preparation of Teeth Prior to Analysis.

The treatment of teeth prior to analysis is dependent to some extent, on the aims of the analysis. Initially, teeth are cleaned. Methods for this include: washing in an ultrasonic bath with distilled water (6), acetone (7), water/pumice slurry (8) and detergent (9); washing with distilled water, silicon carbide slurry (3), dilute detergent solutions (10, 11, 12) and dilute acid (13); soaking in hydrogen peroxide (11, 14), in papain (15, 16) and brushing with bristle or nylon brushes (17, 18, 19). Most of these washing techniques should remove surface dirt, clinging organic matter and blood. However, the more abrasive methods may alter the lead concentration in the surface enamel.

Other techniques have been used to remove tartar, plaque, decayed material, cavities and amalgam fillings. Methods used include: use of dental burr drills (8, 12, 16, 19-26), steel carbide burrs (3, 27, 28), polyethylene and steel scrapers (10, 14, 29), and a pumice scrub (30). Often teeth with cavities or fillings are excluded because of the possibility of contamination from these sources. At this point the whole tooth is ready for

analysis. However, if the investigator is studying the distribution of lead within the tooth, some sectioning would be necessary.

If teeth are to be fractioned further, then two methods are generally applied. The first of these is splitting (31-33) where two notches are cut in the tooth and pressure applied in the hope that the tooth will split cleanly between the two points. This gives a contamination free surface for solid state investigation, but unfortunately, the surface is not smooth. The second method is cutting the tooth along some designated line with a circular saw. Generally water cooled diamond saws (6, 7, 15, 34-48) are used, but silicon carbide (49), slotted steel (3) and carbide steel (27) saws are also used. Parts of the cut tooth can now be removed by chipping (40-42), chiselling (6, 15, 43, 44, 46), filing (50) and reaming (24, 46).

In some investigations of separated enamel and dentine, instead of chipping, the tooth has been crushed followed by densiometric separation in bromoform/acetone mixture. To facilitate this separation the tooth has to be finely powdered. Various methods of crushing have been employed such as a vice (21), agate pestle and mortar (51, 52), alumina pestle and mortar (9) and a "freezer-mill" (23).

The preliminary handling of teeth brings with it the risk of contamination. While the washing techniques are designed to remove contaminating substances, the use of any implements carries the risk of increasing contamination. Nylon and bristle brushes should be reasonably contamination free, but the use of metal implements would possibly cause some surface contamination. This would especially be the

case in crushing mills where a much greater surface area is exposed.

5.2.2 Methods for Dissolution, Concentration and Removal of Interference.

Prior to analysis by atomic absorption spectrophotometry (AAS), anodic stripping voltammetry (ASV) and some of the mass spectrometric methods, the tooth must be dissolved. Dissolution is usually carried out in concentrated nitric acid or a mixture of nitric and perchloric acids. However, these methods do not totally destroy the organic matrix present in teeth. A common method to destroy the organic matrix is the use of dry ashing in a muffle furnace (8, 10-12, 21, 53-56). Ashing temperatures used range from 380^{o}C (12) to 750^{o}C (53), but the most widely chosen temperature is 450^{o}C (10, 11, 54, 55). Whether at these temperatures the ashing is complete is debatable. Albert et al. (53) reported no loss of lead from samples heated to 750^{o}C.

For flame atomisation AAS, preconcentration is necessary principally because of the interference from calcium and phosphate on the lead signal. The most common method employed was the use of an organic complexing agent. This enables a concentrated lead solution to be produced without the presence of high concentrations of calcium and phosphate. The reagent most often used was ammonium pyrollidine-dithiocarbamate (APDC) followed by extraction into methyl isobutyl ketone (MIBK) as the organic solvent (10, 11, 16, 23, 26, 54, 57-63). Other reagents used were

quaternary ammonium bromide in toluene (53, 56), diethyl-dithiocarbamate with ethyl acetate as the organic solvent (64), dithiozone in chloroform (49, 65), and the last method, which is primarily for the removal of interfering species, makes use of selective cation and anion exchange resins (49, 66). It is surprising that in view of the importance of pH on the efficiency of extraction that only one author reports incomplete extraction (41), viz. a 32% loss of lead is reported with an APDC/MIBK system.

For ASV analysis no complexing systems were employed but the solutions obtained from the dissolution of teeth were buffered to reduce the hydrogen ion concentration. Too high a hydrogen ion concentration affects the voltage at which the lead potential wave is observed, and prevents measurement of other ions, such as zinc, in solution.

5.2.3 Methods of Analysis.

The methods of analysis may be divided into two main categories viz. solid state analysis or solution analysis. Most of the solid state analytical methods are based on either the production of X-rays from the lead in the analytical sample, or the production of radioactive isotopes. Contamination from reagents is not a feature of these methods. The wet digestion methods depend generally on using either electric or spectroscopic properties of the analysed element. Reagent selection is important in these analyses. For mass spectrometric methods the sample can be either in solution or in the solid state.

5.2.3 (a) Wet Analytical Methods.

The earliest studies of lead in teeth were by colorimetric analysis where the intensity of colour of a lead complex was used to measure the lead concentration (65). The major problems with this method are the lack of suitable complexes with intense enough absorption bands (required for low lead concentrations), and spectral and chemical interference from other species in solution. The next most widely used early method was DC or AC arc emission spectroscopy (19, 20, 28, 67-70). The major problem with this technique is the high detection limit of around 20ppm (69, 70). However, the limit is dependent on sample size, and some later authors quote lower values.

It would appear that the most popular method for lead determination is AAS. Prior to the late 1970's atomisation was achieved by flames which has two major drawbacks.

(i) The problem of interference and signal suppression: This is particularly a problem for teeth as suppression of the lead signal is caused by the presence of phosphate.

(ii) The problem of sensitivity: To achieve a detection limit of less than 1ppm of lead in a tooth, then the solution concentration necessary will be approximately 10% W/V of tooth. As a consequence high concentrations of calcium in the solution produce high flame noise and background correction is needed. Also, the high concentration of ions can cause blockage of the burner head.

Most authors who employ acid-digestion followed by direct aspiration into a flame AAS use approximately 10% W/V or stronger solutions (8, 25, 29, 47, 55). However, Lappalainen and Knuuttila (51) use a solution of 250mg of tooth powder to 25mL of solution (1% W/V). In this case, assuming a 0.5ppm detection limit, concentrations less than 50 $\mu gPbg^{-1}$ tooth would not be detected, and it is interesting to note that for adults in Finland Lappalainen and Knuuttila (51) have reported an average lead concentration of 53.5 $\mu gPbg^{-1}$ with a range of 30.0 - 79.2$\mu gPbg^{-1}$ for a sample of 124 teeth. This compares with values of 2.4$\mu gPbg^{-1}$ in Norway (17), 2.4$\mu gPbg^{-1}$ in Sweden (71) and 3.8$\mu gPbg^{-1}$ in United Kingdom (57) for adult teeth. Lappalainen and Knuuttila (52) also use a higher concentration of 500mg of tooth in 10mL (5% W/V) and find that dentine lead ranges from 25 - 70$\mu gPbg^{-1}$. However, by using the previous argument their detection limit would be approximately 20$\mu gPbg^{-1}$ in tooth powder.

Two methods are available to combat this problem without changing the analytical method. The first is to use background correction with a lowering of the effective flame noise limits. Wilkinson and Palmer (72) used one tooth (2g) in 50mL and measured levels to only $\pm$1ppm. Their lowest result of 1ppm would require a solution concentration of 0.04ppm which is barely above noise values.

The second solution to this problem is to remove the lead from the interference of calcium and phosphate ions and to raise its concentration in solution. The most frequently employed technique is complexation followed by organic extraction. Various systems have been used (see

Section 5.2.2) but the most common is APDC/MIBK. This allows effective concentrations of one tooth per 1mL (23) to one tooth per 10mL (54) to be used, with an effective 2 - 10 times increase in sensitivity compared with direct aspiration of the acid digested tooth solution.

While it is possible to use flame AAS for whole teeth, in the case of parts of teeth more sensitive methods are necessary. The first improvement was use of the tantalum "boat" accessory for flame AAS (26, 59, 73), which gave some increase in sensitivity. The second improvement was the use of electro-thermal (flameless) AAS with graphite furnaces. The use of graphite furnace atomisation (GFA)-AAS allowed the detection limit to drop to the 1-5ppb range for lead in solution. To some extent the limit is determined by the ability to produce clean enough blanks. The atomisation conditions can be chosen so that calcium and phosphate cause no matrix effect for the lead determination. GFA-AAS has been used to determine lead in whole teeth (57, 71, 74, 75) enamel and dentine (15, 60) and surface enamel (76, 77).

The next most popular method for the analysis of lead in teeth is anodic stripping voltammetry (ASV) (6, 14, 17, 18, 24, 35, 40, 42, 43, 44-45, 46, 57, 78). The accuracy of this method is affected by the levels of contamination, and the problems arising from organic compounds present and high hydrogen ion concentrations. The problem of "organics" can be overcome by dry ashing prior to wet digestion, or using the method of standard additions. The high hydrogen ion concentration can be

overcome by the use of buffers, sodium acetate being the most popular.

5.2.3. (b) X-Ray Emission and Activation Analysis Techniques.

X-ray emission techniques require the bombardment of the sample with a high energy source. Measurement of the emitted X-rays gives an indication of the concentration of analyte in the sample. Of these methods the least successful has been the electron microprobe (EIXE) due to its poor detection limit for lead (approximately 200-1000ppm). Proton induced X-ray emission (PIXE) has been the most widely used of these techniques for determination of lead in teeth (7, 31-33, 37, 38, 79, 80). The detection limit (for lead) varies from 2.5ppm (32, 33) to 21ppm (80) and depends on the proton beam energy. Also the energy of the protons affects the surface penetration of the protons and values of 15-30 μm (31, 80) penetration are reported. One of the drawbacks of this technique is that for a material with a uniform concentration of lead the depth of penetration is not of importance but it can become a problem when looking at surface enamel, where lead concentration varies rapidly with depth. Another probe technique used has been the ion microprobe using O_2^+ and O^-(34). Although spatial deviation is given, no indication of depth penetration is reported by the authors.

By the use of γ-rays from ^{57}Co, Bloch et al. (30, 81) have attempted to measure the X-ray fluorescence of lead in teeth <u>in situ</u>. This relies upon the γ-rays having

a large penetration depth in tooth material. The authors give a detection limit of 15ppm for lead in teeth and find that the results are approximately 20% higher than dentine lead levels obtained by ASV.

The final X-ray method is that of X-ray fluorescence. This has been used to measure lead, among other elements, in powdered dentine. Although the authors (22, 82) quote an error of 10% in their results, they give no idea of their detection limits by this method.

Two techniques of nuclear activation analysis have been employed for looking at lead in teeth. The more widely used method is that of charged particle activation analysis (8, 39, 48, 83-85) generally using the $^{3}He^{2+}$ ion. Irradiation is carried out so that 1ppm of lead is a discernible level. The major problem with this method is the risk of contamination of the surface of a slice of tooth during cutting and polishing. The second technique is neutron activation analysis. In order to remove interference from sodium, calcium, phosphorous and scandium, trace elements were extracted using pyrrolidine-dithiocarbamate and the complex extracted with ethyl acetate. This was used to determine lead and several other elements in tooth enamel (58).

5.2.3 (c) Mass Spectrometric Techniques.

The last group of techniques, that of mass spectrometry offer the advantage of a multi-element technique with the potential to be sensitive enough to look at small areas of a tooth. The first method considered is spark source

mass spectrometry. (SSMS). For this technique, powdered sample is generally mixed with spectrographic graphite to produce an electrode which is used in producing an arc and hence is the source of ions for the mass spectrometer. Limitations on accuracy depend on the purity of the graphite, and elimination of contamination during sample handling. This method has been used to evaluate lead amongst other elements in enamel (2-5, 21, 32) and surface enamel (86).

To reduce the problems of contamination, isotope dilution, high resolution mass spectrometry (IDHRMS) has been used. This method allowed Ericson et al. (49) to measure lead levels down to $0.04 \mu g Pb g^{-1}$ in enamel. For this work, enamel was digested in concentrated nitric/perchloric acid, lead isotopes added and the solution chromatographed on ion exchange resins to remove calcium and phosphate. The lead was then complexed with dithiozone and extracted into chloroform, and finally loaded onto a rhenium filament for thermal ionisation. This method shows the need for preconcentration prior to analysis even with mass spectrometric techniques.

The final spectrographic technique to be used in the investigation of lead concentrations in teeth is secondary ion mass spectrometry (SIMS). This is a surface technique and its unique ability is to quantitatively perform multi-element analysis of the surface while progressively etching into the surface by 0.1 - 1.0μm, with a resolution of 0.05μm. Although not a great deal of work has been done with SIMS on teeth (87-89) it is a method that allows analysis of all areas of a tooth surface or exposed surface.

A summary of the analytical techniques used for lead in teeth is given in Table 5.1

5.2.4 The Problems of Contamination.

Contamination of samples can come from two major areas. The first of these is the reagents used in the analysis. The best that can be done is to minimise these sources of contamination by the use of ultra-pure reagents and repeated distillation of certain reagents, so that the reagents contribution towards the blank value is minimised. Also, it is advisable to make the number of steps from sample to final analysis as small as possible and the number of reagents added should be kept to a minimum. However, balanced against this is the need, with some methods of analysis, to preconcentrate the lead to a level where instrumental noise is no longer a problem.

The other area of contamination is in the cutting, filing, grinding and polishing of samples required for some methods of analysis. A reported case of possible contamination with lead was from a drill used to obtain dentine samples (22, 82). While contamination has been reported for other elements where saws have been used to section teeth, some studies which use a splitting technique give an uncontaminated surface for their surface studies (31-33, 89).

Therefore while reagent purity is a problem with digested samples, surface contamination becomes a problem with surface analytical techniques. Unfortunately many authors do not clearly state their estimated contamination

Table 5.1

Analytical Methods Used for Lead in Teeth.

Method	Reference
Anodic Stripping Voltammetry (ASV)	6, 14, 17, 18, 24, 35, 40-46, 57, 78, 90
Flame Atomisation Atomic Absorption Spectrophotometry (flame-AAS)	8-11, 13, 16, 23, 25, 29, 47, 51-57, 61-64, 66, 72, 78, 91-95
Tantalum-Boat Atomic Absorption Spectrophotometry ("Boat"-AAS)	26, 59, 73
Graphite Furnace Atomic Absorption Spectrophotometry (GFA-AAS)	15, 57, 60, 71, 74-77
Spectrographic	19, 20, 28, 67-70
Colorimetric	65
Polarograph	12
Neutron Activation Analysis (NAA)	58
Charged Particle Activation Analysis (CPAA)	39, 48, 83-85

Table 5.1 cont.

Method	Reference
Electron Microprobe (EIXE)	36
Proton-Induced X-ray Emission (PIXE)	7, 31-33, 37, 38, 79, 80
Ion-Induced X-ray Emission (IIXE)	34
γ-ray Induced X-ray Emission	30,81,96
X-ray Fluorescence (XF)	22, 82
Spark Source Mass Spectroscopy (SSMS)	2-5, 21, 27, 86
Second Ion Mass Spectroscopy (SIMS)	87-89
Isotope Dilution, High Resolution Mass Spectroscopy (IDHRMS)	49

levels and it is often not possible to make estimates from the data published.

5.3.1 Discussion

In order to discuss the results of previous work on lead concentrations in teeth it is necessary to consider data for a similar type of sample and then to comment on the effects of various factors that affect the measured values. The Tables 5.2 - 5.9 given at the end of this discussion are drawn up for permanent and deciduous teeth, separately, sub-divided into the various zones of tooth analysed, and sometimes into the type of tooth analysed. The factors that will be considered are: the effects of age and sex, the distribution of lead within the tooth, the variation in lead levels with tooth type, the effect of geographical variations, and finally a comparison between ancient and modern teeth in order to arrive at natural background levels.

5.3.2 The Effect of Age on Tooth Lead Concentrations.

If teeth are to be used as an indicator of lead exposure, and since it is assumed that lead accumulates in teeth, then it would seem that the concentration of lead should increase with the age of a person. However, if, after eruption, the tooth undergoes no further change then the lead levels would merely reflect the levels at the time of tooth formation and no age effect would be seen.

The evidence obtained on whole permanent teeth suggests that the lead concentration increases with the age of the donor (39, 51, 52, 56, 68, 72). Steenhout and Pourtois (55) correlate lead concentration with tooth age for three populations in Belgium and obtain regression coefficients ranging from 0.93 to 0.99, suggesting that tooth age is a better parameter to correlate with lead concentration than donor age. The circumpulpal dentine has been shown to increase in lead concentration with donor age (39, 83, 85), probably because this tissue is in direct contact with the blood system and is being continuously laid down. The dentine also shows an increase in lead concentration with donor age (39, 52, 73, 83, 91), and may suggest that there is some mobility of lead ions in dentine. Evidence for higher lead levels in enamel with age is not so clear. In fact several authors (39, 52, 83, 85, 91) suggest that bulk enamel is relatively static as regards lead concentration, but Brudevold and Steadman's (67) work suggests that enamel lead levels increase with age. Since tooth enamel is a more stable and dense matrix, ionic mobility may be low, making the enamel lead practically inert. Surface enamel lead concentrations do, however, appear to show an age effect (39, 67), and this could be linked to ion-exchange and isomorphous replacement processes occurring on the surface.

For deciduous teeth the evidence is strongly suggestive of a positive age/lead concentration relationship (16, 26, 56, 74, 81, 97). Fergusson et al. (15) however, found no significant age effect for lead concentrations in dentine. This may be due to the fact that their deciduous teeth were

obtained as shed teeth from donors. In this case the actual tooth age could well be similar for all teeth, although the donor age will vary with tooth type. Brudevold et al. (77) found that surface enamel tended to decrease with donor age.

5.3.3 The Effect of Donor Sex on Lead Concentration in Teeth.

There is no evidence to suggest that there is an effect due to donor sex on lead concentration. For permanent teeth various authors have found no significant sex related differences in lead concentration in teeth (52, 64, 68, 72, 91). Similarly in deciduous teeth, various authors have found no significant effect due to sex on lead levels in teeth (11, 16, 72, 95).

5.3.4 The Distribution of Lead in Teeth.

The distribution of lead is not uniform within the various parts of a tooth. The main zones investigated have been surface enamel, bulk enamel, the amelo-dentine junction, dentine and circumpulpal dentine.

For permanent teeth the highest levels of lead are found in surface enamel and circumpulpal dentine (31, 39, 48, 89). An explanation for this is that the exterior exposed surfaces of the tooth and the pulpal cavity are the only areas of the tooth that are exposed to lead directly. The lead concentration in surface enamel decreases very rapidly with depth (67, 76, 77, 89), suggesting that lead has been

absorbed and then incorporated, by isomorphous replacement of calcium, in the apatite matrix. It is believed that dentine is continuously being laid down in the pulpal cavity, but at a reduced rate once the tooth has erupted. This could account for the high lead levels found in circumpulpal dentine compared with primary dentine (24, 39, 48, 83, 85, 89).

For permanent teeth it is suggested that there can be some movement of lead ions within dentine, and that lead slowly migrates from the circumpulpal dentine into the primary dentine. (See Section 5.3.2). This effect may influence the lead concentration in dentine relative to enamel. It appears that in young permanent teeth, enamel lead concentrations tend to be higher than dentine lead concentrations, but as the donor grows older this is reversed with the dentine lead concentrations being the higher (39, 52, 91). In other studies however, dentine lead levels have been found to be always higher than enamel lead concentrations (24, 38, 48, 70, 83, 85).

The final zone of permanent teeth to be investigated is the amelo-dentine junction (ADJ). Malik and Fremlin (39) report a pronounced peak in lead concentration at the ADJ, while other authors have found that at the ADJ the lead concentration shows a smooth transition from the lead concentration in enamel to the lead concentration in dentine (48, 89).

Data for lead concentration in various zones of permanent teeth is summarised in Tables 5.3 and 5.4

Similar patterns of lead distribution in deciduous teeth have been found to those obtained for permanent teeth.

The highest lead concentrations are found on surface enamel (24, 89) and in circumpulpal dentine (24, 45, 89). Differences between the lead concentrations in enamel and dentine are, however, more confused. It would appear that the ratio of lead concentration in enamel to the lead concentration in dentine is dependent on the donor's level of exposure to lead. If the level of exposure has been low then the lead concentration in enamel is higher (24, 25, 60, 61). However, if the exposure to lead has been high then the lead concentration in dentine is higher than the lead concentration in enamel (13, 59, 89).

For deciduous teeth the relative position of the amelo-dentine junction (ADJ) lead concentration is also not clear. Carrol et al. (36) using an electron microprobe found lead to be concentrated in the "hypomineralised" zones, that is, the circumpulpal dentine and the ADJ. However, Petersson et al. (89) using second ion mass spectroscopy found a smooth change in lead concentration going through the ADJ from the enamel to the dentine.

Data for lead levels in various zones of deciduous teeth is summarised in Table 5.6 and 5.7.

5.3.5 Intra-mouth Variability in Tooth Lead Concentration.

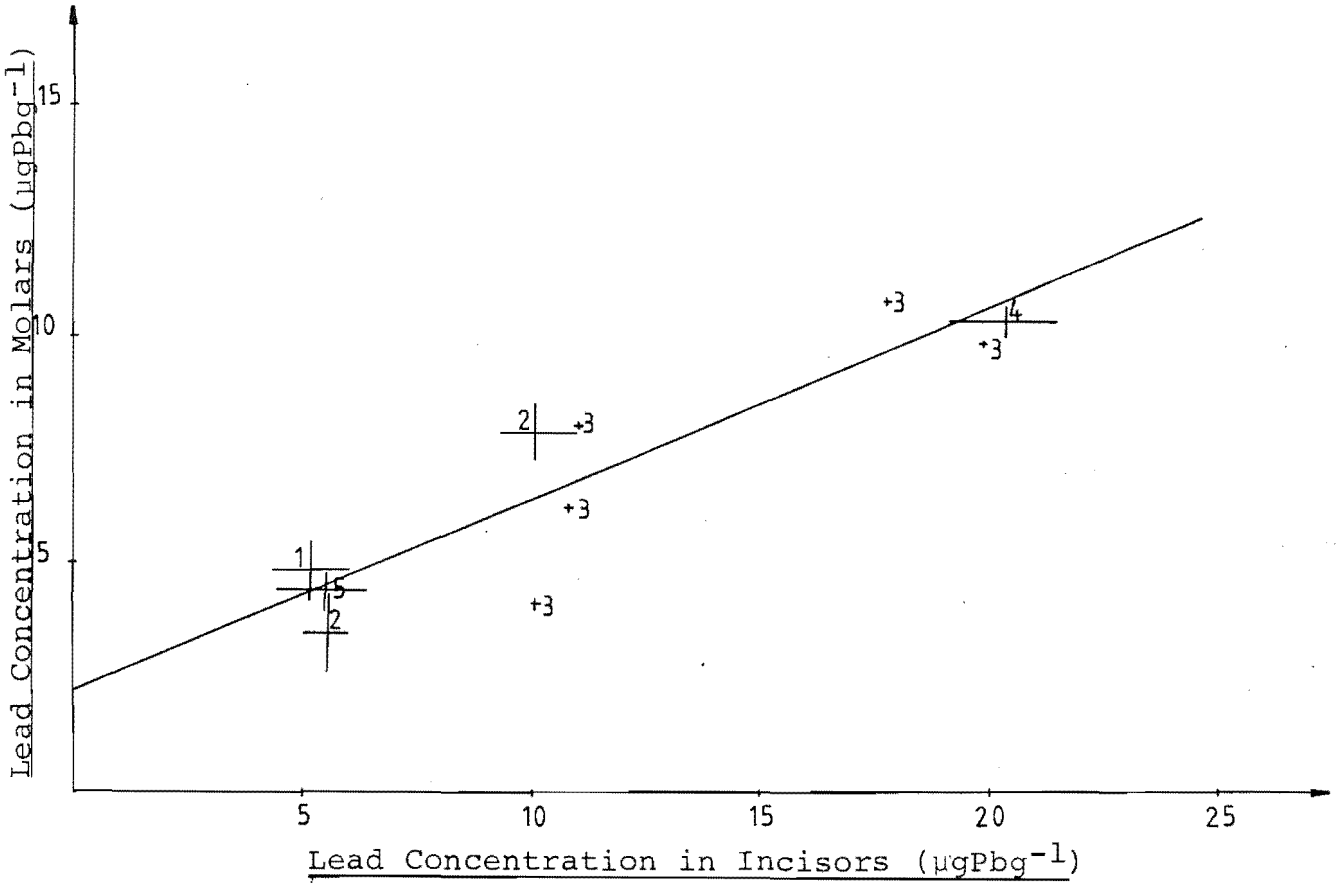
From the literature it would appear that the variability of lead concentration with the type of tooth is partly a factor of tooth age, as opposed to donor age. For deciduous teeth where the order of tooth eruption is incisors, canines, molars, it has been found that lead

concentrations in incisors are higher than canines which are higher than molars, (9, 10, 15, 16, 24, 45, 78, 94, 95). In some cases however, no significant difference was found (35, 53, 59, 73) between incisors and other tooth types. There appears to be no significant difference between upper and lower jaw values (35) although this has not been widely studied. In summary, it would appear that for deciduous teeth there is a significant intra-mouth variability with the most marked difference being between incisors and molars. (See Figure 5.1 and Tables 5.8 and 5.9).

For permanent teeth the pattern of intra-mouth variability is not so clear. Firstly, the order of eruption is approximately central incisors, first molars, lateral incisors, canines, premolars, second molars and third molars. This compounds the problem of teeth classification, so that if levels are dependent on tooth age then a simple incisor, canine, premolar, molar split is not adequate. A second factor is that there are very few reports which give lead concentrations for all the various categories of permanent teeth. While it would be tempting to extrapolate the results of deciduous teeth to permanent teeth there is yet insufficient evidence to do so. Some authors have, however, noted differences between various tooth types for permanent teeth (60, 93). Again, there is no evidence to suggest there is any significant difference between teeth of the upper and lower jaw.

Figure 5.1

Graph Showing Relationship of Lead in Incisors to Lead in Molars for Deciduous Teeth.



Key:

No.	Ref.
1	10
2	15
3	9
4	16, 95
5	78

5.3.6 Geographical Variation of Lead Concentration in Teeth.

The levels of lead in teeth have been used to indicate different environmental exposures of the tooth donors to lead. Because of the considerable interest in the subclinical effects of lead poisoning in children, and the use of deciduous teeth lead concentrations as a marker of previous lead exposure to the donor, considerable data is available on the geographical variables which affect lead teeth concentrations.

The main variables that have been noted for deciduous teeth are (a) residence in an industrialised zone (13, 15, 26, 40-42, 59, 62, 72, 73, 97, 98) especially near a non-ferrous smelter or lead works (74, 75); (b) the condition of housing, especially the presence of lead paint (9, 15, 23, 35, 42) or lead water piping (12); (c) whether the donor lives in urban, suburban or rural environments (10, 11, 16, 45, 46). Considerable interest and dispute have been aroused over whether lead from petrol plays a significant role in increased tooth-lead concentrations. Needleman et al. (40, 41) have suggested that lead from petrol does play a significant role in increased body burdens of lead, Kelsall and Hunter (8) on the other hand demonstrated a correlation between tooth lead levels and air lead levels, but not with traffic density or the existence of old housing. However, it has not been conclusively demonstrated as to what contribution automotive lead plays in the increased lead burdens of people living in urbanised communities.

For permanent teeth similar factors have been used to explain the differences in lead concentrations in teeth from different geographical areas. Variation between urban and rural environment has been noted (33, 46, 52, 54, 72). Sheenhout and Pourtois (55) note that the presence of a non-ferrous smelter has a more significant effect on lead levels in teeth, than living in an urban centre. This work (55) also shows the importance of age matching of populations when making comparisons between different environments.

Lappalainen and Knuuttila (51) attempted to find a correlation between soil lead levels and the tooth-lead concentrations of the population but no significant correlation was found.

One of the most interesting studies was Shapiro et al.'s (46) study of lead in circumpulpal dentine of people from Philadelphia and Mexican Indians living in the Lacandon Forest of Chiapas, Mexico, which showed that teeth from Philadelphia had approximately 45 times more lead in the circumpulpal dentine then the Mexican Lacandon and Tzeltales Indians.

5.3.7 Comparison of Lead Concentrations in Ancient and Modern Teeth.

Investigation of the lead concentration in ancient teeth has been directed at establishing an estimate of how much greater present day exposure to lead is compared with some time in the past. The work of Ericson et al. (49) suggests that compared with ancient Peruvians modern levels are between 200 and 700 times higher today. Grandjean et al.

(50) measured lead in circumpulpal dentine of Nubian people from the Sudan who had died around 5000 years ago and found that modern peoples have approximately 200 times higher lead concentrations.

In more modern times, from about 0 A.D., lead has been used in glass, pipeworks, pewter and as a preservative for wine. This effect is demonstrated by the higher levels in ancient teeth compared with contemporary teeth (18, 57). Taken overall the literature is suggestive of a slowly increasing level of lead over the last 2000-3000 years as man's use of lead has increased. (18, 22, 46, 49, 50, 57, 59, 72, 73, 82). (See Figure 5.2).

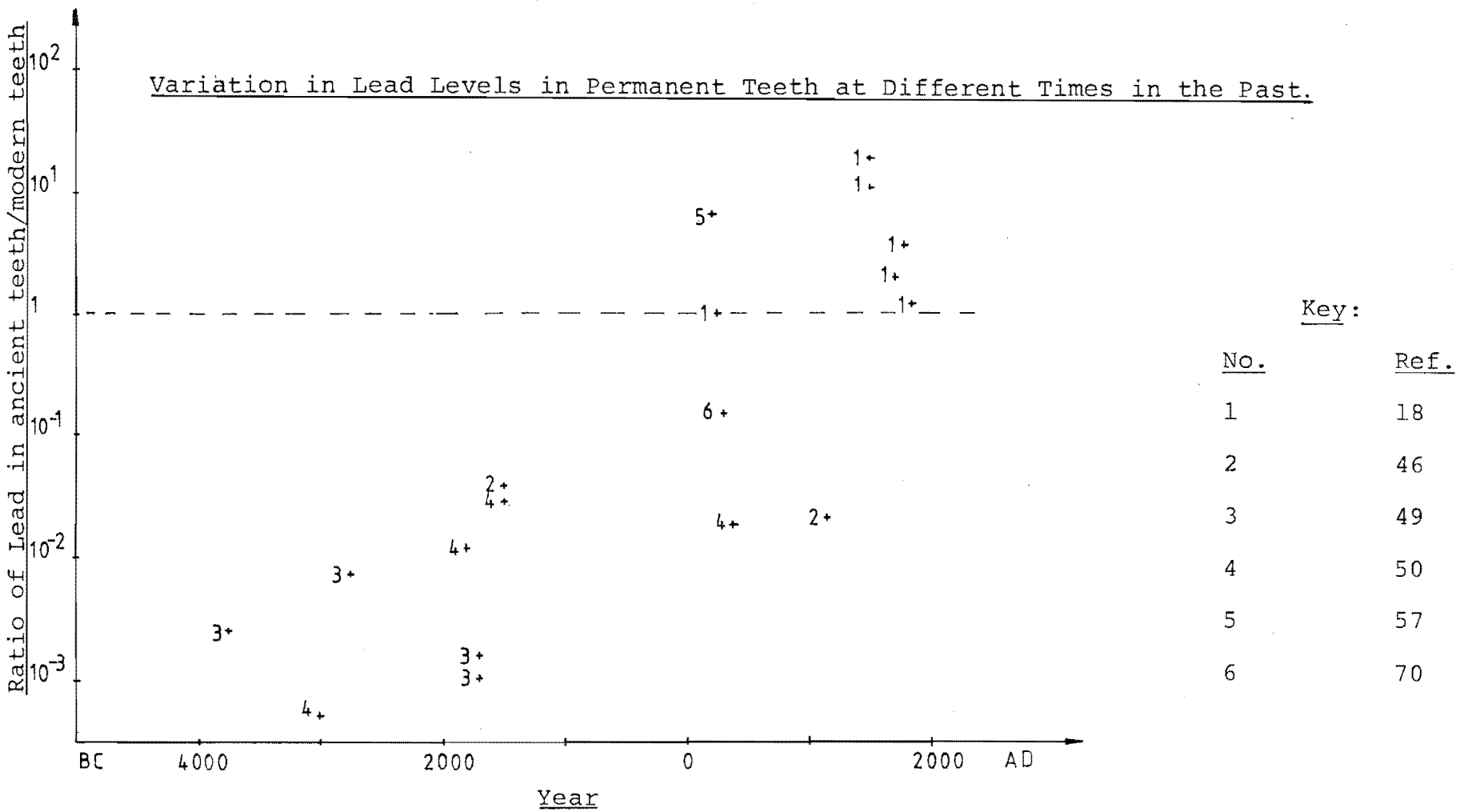
5.4.1 Summary

From a survey of previous work the following conclusions can be drawn:

(1) That the concentration of lead increases with age. Therefore tooth material can be used as a cummulative indicator of past lead exposure to the tooth donor. The only exception to this is that bulk enamel is more stable than the rest of the tooth and the lead concentration in bulk enamel reflects more the lead exposure at the time of tooth formation.

(2) That the concentration of lead varies with the type of tooth. Although the evidence for this is reasonably conclusive for deciduous teeth, the effect of tooth age complicates the picture for permanent teeth.

Figure 5.2


Variation in Lead Levels in Permanent Teeth at Different Times in the Past.
Ratio of Lead in ancient teeth/modern teeth
10^2
10^1
1
10^{-1}
10^{-2}
10^{-3}
BC
4000
2000
0
2000
AD
Year
Key:
No. Ref.
1 18
2 46
3 49
4 50
5 57
6 70

(3) That the concentration of lead varies within a tooth. The highest levels of lead are found on the exterior surface enamel and in circumpulpal dentine. The ratio of lead concentration in enamel to lead concentration in dentine is dependent on tooth age for permanent teeth and for deciduous teeth on the level of exposure to lead. The balance of the evidence suggests that the amelo-dentine junction shows a smooth transition from enamel to dentine in lead concentration.

(4) That the sex of the donor has no effect on lead concentration.

(5) That the concentration of lead in teeth is dependent upon the amount of lead in the environment. Lead concentration in teeth has been shown to be dependent on the age and state of housing, on the use of lead water piping, on the presence of industrial output of lead, on the lead concentrations in air and the presence of automotive lead.

(6) That the concentration of lead in modern teeth is higher than in teeth from pre-industrialised periods. The only exceptions to this are periods in history when lead was used in utensils and the piping of water.

(7) That the presence of lead in enamel has little effect on caries prevalence.

5.4.2 Use of Teeth in Surveys of Population Exposure to Lead.

If teeth are to be used in studies of lead exposure

to a population then the following factors should be considered:

(1) When choosing a material for the study, it is best to avoid enamel as it may not reflect accumulated lead dosage to the donor. Dentine, circumpulpal dentine and whole teeth on the other hand do appear to reflect accumulated lead exposure.

(2) When comparing two or more populations, the lead concentrations should be normalised for tooth age. Also the populations should contain a similar mix of teeth or preferably only one type of tooth, the best probably being central incisors as these appear to have the highest lead concentrations.

(3) When carrying out a survey, the method of analysis must be sufficiently sensitive and any interferences that may exist removed or allowed for. This requirement is to allow comparisons to be made with other studies. Also the use of an internal standard is advised so that contamination may be checked.

Table 5.2

Lead Concentration in Whole Teeth (in $\mu gPbg^{-1}$ dry weight).

Location	Mean	Median	Range	S.D.	No.	Year[1]	Reference	Analytical[4] Method
Oslo, Norway	3.06		1.8-4.9	1.06	10	1978	14	ASV
Oslo, Norway	2.4		0.9-7.8		44	1976	17	ASV
Bolstad (Gjerpen) Norway	2.5		2-3	0.5	6	200-300AD	18	ASV
Gimsøy Kloster Norway	28		8-45	19	3	1500AD	18	ASV
Petrikirken Tønsberg, Norway	49		15-140	47	6	1500AD	18	ASV
Tingvoll, Kirke Norway	9		5-15	5	3	1780AD	18	ASV
Årøen, Alta Norway	3		3-3	0	3	1830AD	18	ASV
Bjolstad Kirke, Heidal, Norway	5		1-15	6	5	1700AD	18	ASV

Table 5.2 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Finland[2]	53.5		30-79.2	7.8	124	1979	51	AAS
Japan	7.29		1.12-64.67	+10.12 -4.22	93	1972	54, 93	AAS
Tokyo, Japan	11.61		3.93-46.93	+12.74 -6.07	24	1974	54	AAS
Okitsu, Japan	4.65		1.60-13.27	+4.01 -2.15	20	1974	54	AAS
Hachijo, Is., Japan	8.64		1.12-64.67	+17.00 -5.73	17	1974	54	AAS
Annaka, Japan	6.27		1.37-31.15	+7.10 -3.33	35	1974	54	AAS
Hoboken, Belgium[3]	35.4		5.4-110.7	6.0	51	1981	55	AAS
Brussels, Belgium[3]	17.7		2.3-68.0	4.2	42	1981	55	AAS
Arlon, Belgium[3]	21.5		1.2-69.3	4.4	38	1981	55	AAS
U.K.		3.8	2-8		37	1980	57	GFA-AAS +B/G

Table 5.2 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
U.K.		80				Romano-British	57	GFA-AAS +B/G
Bombay, India		15.5	4.27-82.5			1978	64	AAS
Sweden	2.4		1.1-6.4	1.5	14	1974	71	GFA-AAS
Newcastle on Tyne, U.K.	2.41		1.22-3.60	0.83	8	1978	78	ASV
Harrisburg, Pennsylvania, USA.	16				5	1952	99	

Notes: (1) Year of publication, unless date of collection or date of tooth known.

(2) Chemical Abstract only obtained.

(3) Ash weight, not dry weight (Ash weight = dry weight - 21±3%).

(4) Unless otherwise specidied, AAS is assumed to be flame atomisation.

(5) S.D. means standard deviation.

(6) No. is the size of the sample in the study.

Table 5.3

Lead Concentration in Various Parts of Permanent Teeth (in $\mu gPbg^{-1}$ dry weight).

Section A: Surface Enamel

Location	Mean	Median	Range	S.D.	Depth (μm)	No.	Year	Reference	Analytical Method
Watertown, N.Y., USA. (<30yr.)	70				60	45	1966	19	Spectrographic
Watertown, N.Y., USA. (>30 yr.)	110				60	70	1966	19	Spectrographic
Sweden	140				≈30	1	1976	31	PIXE
"Acid susceptible" USA.	2		0-7		surface	4	1975	34	SIXE
"Acid resistant" USA.	1.9	0-7	0-7		surface	4	1975	34	SIXE
Birmingham, U.K.	40		30-55	10		6	1974	39	CPAA
Birmingham, U.K.			100-150			2	1974	48	CPAA

Table 5.3 cont.

Location	Mean	Median	Range	S.D.	Depth (μm)	No.	Year	Reference	Analytical Method
Rochester, N.Y., USA.			180-550				1956	67	Spectrographic
Pueblo Indian, USA.	80					12	919-1130	70	Spectrographic
Indian Knoll, USA.	85					23	3000BC	70	Spectrographic
Cambridge, Mass., USA.	1790		200-3550	960	2.7±0.7	35	1975	76	GFA-AAS
Scandinavia			21-43		15	2	1980	80	PIXE
All over the world	24	18	1.2-79		42±8.5	54	1979	86	SSMS
Sweden			300-2000		0.1	2	1978	89	SIMS

Section B: Enamel

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
USA.	5.9			4.3	335	1976	2	SSMS

Table 5.3 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
USA.	19.63		0.01-270		335	1977	4	SSMS
USA.	19.64		0.0-156		451	1978	5	SSMS
Watertown, N.Y. USA. (<30 yr.)	30				41	1966	19	Spectrographic
Watertown, N.Y. USA. (>30 yr.)	85				96	1966	19	Spectrographic
Manchester, U.K.	10				2	1967	21	SSMS
Pennsylvania, USA.	36		13-70	20	7	1972	24	ASV
USA.	3.1	2.9	1.0-6.5	0.16	56	1974	27	SSMS
USA.	8.5	4.4	2-56	1.21	93	1974	28	Spectrographic
Sweden	50				1976	31	PIXE	
"Acid susceptible" USA.	0.8				4	1975	34	SIXE
"Acid resistant" USA.	1.2				4	1975	34	SIXE

Table 5.3 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
New South Wales, Australia	10				15	1981	38	PIXE
Birmingham, U.K.	34.2		24.5-53.5	10.3	7	1974	39	CPAA
Birmingham, U.K.	≈60				2	1974	48	CPAA
Peru	0.10					4500-3000 BC	49	IDHRMS
Peru	0.23					3000-2500 BC	49	IDHRMS
Peru	0.04					2000-1400 BC	49	IDHRMS
Peru	0.06					2000-1400 BC	49	IDHRMS
Rural, Eastern Finland.	64.3			16.1	89	1981	52	AAS
Kuopio, Urban Finland.	56.6			11.0	50	1981	52	AAS

Table 5.3 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Pueblo Bonito, New Mexico, USA.	12				12	919-1130	70	Spectrographic
Indian Knoll, USA.	60				23	3000BC	70	Spectrographic
Birmingham, U.K.	2.5		1.4-5.3	1.0	29	1980	83	CPAA
Sheffield, U.K.	3.1		1.2-5.4	1.7	16	1980	83	CPAA
Aberystwyth, U.K.	2.0		1.6-2.9	0.6	15	1980	83	CPAA
Birmingham, U.k.	2.0		1-5		25	1980	85	CPAA
Sheffield, U.K.			1-5		16	1980	85	CPAA
Sweden			150-200		2	1978	89	SIMS
Virginia, USA. (10-12yr,)	43.2			1.0	39	1974	91	AAS
Virginia, USA. (13-16yr.)	43.6			0.9	47	1974	91	AAS
Virginia, USA. (17-24yr.)	45.1			0.9	43	1974	91	AAS

Table 5.3 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Virginia, USA. (>25yr.)	48.8			0.9	44	1974	91	AAS

Section C: Dentine

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Manchester, U.K.	≃8				2	1967	21	SSMS
Pennsylvania, USA.	52		26-97	28	7	1972	24	ASV
Sweden	8.9				1	1976	31	PIXE
Malmö, Sweden	4.5			3.5	26	1976	32	PIXE
Stockholm, Sweden	3.4			1.4	38	1976	32	PIXE
Jonkoping, Sweden	3.2			1.3	23	1976	32	PIXE
New South Wales, Australia			10-30		15	1981	38	PIXE

Table 5.3 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Birmingham, U.K.	33.1		25.6-46	9.0	7	1974	39	CPAA
Birmingham, U.K.	≈60				2	1974	48	CPAA
Rural, Eastern Finland	≈41		25-70		89	1981	52	AAS
Kuopio, Urban Finland	≈53		30-90		50	1981	52	AAS
Pueblo Bonito, New Mexico, USA.	28		13-44	13	12	919-1130	70	Spectrographic
Indian Knoll, USA.	64		60-67		23	3000BC	70	Spectrographic
Birmingham, U.K.	13.8		5.0-39.6	7.3	29	1980	83	CPAA
Sheffield, U.K.	19.9		3.8-66.7	12.4	16	1980	83	CPAA
Aberystwyth, U.K.	17.3		4.2-81	22.8	15	1980	83	CPAA
Birmingham, U.K.			4-40		28	1980	85	CPAA
Sheffield, U.K.			3-38		17	1980	85	CPAA
Sweden			50-120		2	1978	89	SIMS

Table 5.3 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Virginia, USA.	43.4			5.6	175	1974	91	AAS

Section D: Circumpulpal Dentine

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Pennsylvania, USA.	182		122-320		6	1972	24	ASV
Sweden	29				1	1976	31	PIXE
Philadelphia, USA.	188.3	175.0	122.1-233.4	37.9	6	1975	46	ASV
Alaska, North Slope, USA.	56.0	47.1	9.6-97.8	30.1	7	1975	46	ASV
Manchu Picchu	13.6	3.7	0.2-56.1	19.8	7	1100-1200	46	ASV
Egypt	9.7	6.9	0.6-29.8	10.7	7	1st & 2nd millenia	46	ASV
Birmingham, U.K.	≃150				2	1974	48	CPAA

Table 5.3 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Nubian, Sudan		0.9	0.4-1.4		9	3300-2900 BC	50	ASV
Nubian, Sudan		2.1	0.9-3.1		13	2000-1600 BC	50	ASV
Nubian, Sudan		5.0	2.4-7.7		15	1650-1350 BC	50	ASV
Nubian, Sudan		3.2	2.4-4.4		68	1-750AD	50	ASV
Denmark		25.7	17.0-42.4		17	1979	50	ASV
Birmingham, U.K. (12-16yr.)	34.2		17.3-43.9	17.0	10	1980	83	CPAA
Birmingham, U.K. (40-71yr.)	92.2		21.0-213.1	31.9	19	1980	83	CPAA
Sheffield, U.K. (12-16yr.)	18.9		12.5-24.5	7.4	5	1980	83	CPAA
Sheffield, U.K. (40-72yr.)	112.5		28.2-299.6	52.9	11	1980	83	CPAA

Table 5.3 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Aberystwyth, U.K. (5-15yr.)	27.5		20.8-54.3	9.6	10	1980	83	CPAA
Aberystwyth, U.K. (40-70yr.)	149.3		112.6-310.3	92.5	5	1980	83	CPAA
Birmingham, U.K.			18-175		30	1980	85	CPAA
Sheffield, U.K.			12-155		18	1980	85	CPAA
Sweden			200-500		2	1978	89	SIMS

Notes: (1) S.D. means standard deviation.

(2) No. is the size of the sample in the study.

(3) Unless otherwise specified, AAS is assumed to be flame atomisation.

Table 5.4

Comparison of Lead Levels in Various Parts of Permanent Teeth (in $\mu gPbg^{-1}$ dry weight).

Location	Surface Enamel	Bulk Enamel	A.D.J	Dentine	Circumpulpal Dentine	No.	Year	Ref.	Analytical Method
Watertown, NY, USA (<30yr.)	70	30				41	1966	19	Spectrographic
Watertown, NY, USA (>30yr.)	110	85				70	1966	19	Spectrographic
Manchester, U.K.		≃10		≃8		2	1967	21	SSMS
Pennsylvania, USA.		36±2.0		52±28	190±70	7	1972	24	ASV
Sweden	140	50		8.9	29	1	1976	31	PIXE
USA.	2.0	1.0				8	1975	34	Ion Microprobe
New South Wales, Australia		10		10-30		15	1981	38	PIXE
Birmingham, U.K. (19yr.)	33.8±1.7	27.4±1.6		26.7±1.0	26.3±1.0	1	1974	39	CPAA
Birmingham, U.K. (19yr.)	33.6±1.5	25.7±1.2		29.3±0.6	26.8±0.8	1	1974	39	CPAA

Table 5.4 cont.

Location	Surface Enamel	Bulk Enamel	A.D.J.	Dentine	Circumpulpal Dentine	No.	Year	Ref.	Analytical Method
Birmingham, U.K. (21 yr.)	31.1±1.8	22.8±1.4		25.0±1.0	27.8±1.4	1	1974	39	CPAA
Birmingham, U.K. (25 yr.)	55.0±2.0	52.5±2.1		38.5±1.9	49.8±2.0	1	1974	39	CPAA
Birmingham, U.K. (27 yr,)	47.5±1.3	34.1±1.2		27.4±1.0	32.3±1.2	1	1974	39	CPAA
Birmingham, U.K. (43 yr.)	38.5±1.7	29.4±1.7		41.0±1.8	50.6±1.9	1	1974	39	CPAA
Birmingham, U.K.	110	60	75	90	130	1	1974	48	CPAA
Birmingham, U.K.	150	80	90	110	150	1	1974	48	CPAA
Rural Finland		64.3±16.1		≃41		89		52	AAS
Urban Finland		56.6±11.0		≃53		50		52	AAS
Pueblo Bonito, New Mexico, USA.	80	12		24±10		12	1959	70	Spectrographic
Birmingham, U.K. (12-16 yr.)		2.3±1.0 (1.6-3.6)		7.2±3.4 (5.8-12.6)	34.2±17.0 (17.3-43.9)	10	1980	83	CPAA

Table 5.4 cont.

Location	Surface Enamel	Bulk Enamel	A.D.J.	Dentine	Circumpulpal Dentine	No.	Year	Ref.	Analytical Method
Birmingham, U.K. (40-71 yr.)		2.7±0.7 (1.4-5.3)		17.3±7.3 (5.0-39.6)	92.2±31.9 (21.0-213.1)	19	1980	83	CPAA
Sheffield, U.K. (12-16 yr.)		2.1±1.0 (1.4-3.5)		7.2±2.9 (3.8-10.4)	18.9±7.4 (12.5-24.5)	5	1980	83	CPAA
Sheffield, U.K. (40-72 yr.)		4.0±0.7 (1.2-5.4)		25.6±12.4 (5.5-66.7)	112.5±52.9 (28.2-299.6)	11	1980	83	CPAA
Aberystwyth, U.K. (5-15 yr.)		2.0±0.6 (1.6-2.9)		5.6±2.0 (4.2-11.1)	27.5±9.6 (20.8-54.3)	10	1980	83	CPAA
Aberystwyth, U.K. (40-70 yr.)				40.6±22.8 (26.9-81.0)	149.3±92.5 (112.6-310.3)	5	1980	83	CPAA
Birmingham, U.K.		1-5		4-40	18-175		1980	85	CPAA
Sheffield, U.K.		1-5		3-38	12-155		1980	85	CPAA
Sweden (high fluoride)	≈600	≈150	≈100	≈100	≈500	1	1978	89	SIMS
Sweden (low fluoride)	≈300	≈90	≈50	≈120	≈200	1	1978	89	SIMS

Table 5.4 cont.

Location	Surface Enamel	Bulk Enamel	A.D.J.	Dentine	Circumpulpal Dentine	No.	Year	Ref.	Analytical Method
Virginia, USA. (10-12 yr.)		43.2±1.0		38.9±1.4		39	1974	91	AAS
Virginia, USA. (13-16 yr.)		43.6±0.9		42.2±1.2		47	1974	91	AAS
Virginia, USA. (17-24 yr,)		45.1±0.9		40.7±1.3		43	1974	91	AAS
Virginia, USA. (>25yr.)		48.8±0.9		51.5±1.3		44	1974	91	AAS

Notes: (1) Ref. means reference.

(2) A.D.J. means amelo-dentine junction.

(3) No. is the size of the sample in the study.

(4) Unless otherwise specified, AAS is assumed to be flame atomisation.

(5) Values in table are mean±standard deviation with range in brackets below these values.

(6) Year of publication, unless date of collection or date of tooth known.

Table 5.5

Levels of Lead in Whole Deciduous Teeth (in $\mu g Pb g^{-1}$ dry weight).

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Cleveland, USA. (Urban)	55.7			20.5	5333	1978	8	AAS
Cleveland, USA. (Suburban)	54.5				3704	1978	8	AAS
St. Louis, USA. (lead paint zone).		15.3			115	1975	9	AAS
St. Louis, USA. (public housing).		17.5			29	1975	9	AAS
St. Louis, USA. City (no lead paint).		8.4			72	1975	9	AAS
St. Louis, USA. Suburban		10.0			28	1975	9	AAS
St. Louis, USA. Industrialised		8.9			43	1975	9	AAS

Table 5.5 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Tennessee, USA.	5.0		1-60	5.9	153	1975	10	AAS
Enfield, U.K.[1]	4.7				1243	1982	11	AAS
Hounslow, U.K.[1]	5.1				395	1982	11	AAS
London, U.K.[1]	4.1				195	1982	11	AAS
Reading, U.K.[1]	4.4				83	1982	11	AAS
Birmingham, U.K.[1]	4.8				70	1982	11	AAS
Hammersmith, U.K.[1]	5.5				36	1982	11	AAS
Manchester, U.K.[1]	6.3				11	1982	11	AAS
Berks./Harts., U.K.[1]	3.6				103	1982	11	AAS
Glasgow, Scotland. (new housing)	8.4			5.5	39	1978	12	Polarograph
Glasgow, Scotland (old housing)	20.3			8.6	19	1978	12	Polarograph
Bothasig, S.A.	20419			3429	21	1982	13	AAS
Parowvallei, S.A.	16556			6940	27	1982	13	AAS

Table 5.5 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Birmingham, U.K. Industrial	11.4			6.10	544	1977	16	AAS
Birmingham, U.K. Suburban	11.6			7.20	617	1977	16	AAS
Sutton, Coldfield. U.K.	9.9			6.22	60	1977	16	AAS
Wednesfield, U.K.	10.2			3.55	83	1977	16	AAS
Birmingham, U.K.	11.8	10.4			1392	1976	16, 95	AAS
Oslo, Norway	5.6		1.5-13.4		32	1976	17	ASV
Cincinnati, Ohio, USA	15.1		1.3-57	11.6	82	1962	20	Spectrographic
Philadelphia, USA. ("lead belt")	51.1		2-650	109.0	69	1972	23	AAS
Philadelphia, USA. Suburban	11.1		BDL-70	14.8	40	1972	23	AAS
Belfast, Ireland. Urban	7.54				148	1974	26	"Boat"-AAS

Table 5.5 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Belfast, Ireland Suburban	6.16				91	1974	26	"Boat"-AAS
County Fermanagh, Ireland. Rural	4.12				71	1974	26	"Boat"-AAS
Severnside, U.K.	30.2			7.1	20	1957-63	29	AAS
Severnside, U.K.	36.0			5.3	20	1972-73	29	AAS
Tyneside, U.K.	36.8			7.5	10	1973	29	AAS
New York City, USA.[1,2]		11.5	2-60	2.8	626	1974	53	AAS
Norway	3.73			4.98	2233	Modern	59, 73	"Boat"-AAS
Busderud County, Norway	4.12			3.08	273	Modern	59, 73	"Boat"-AAS
Bryggen, Norway	1.81			0.60	24	Medieval	59, 73	"Boat"-AAS
Duisberg, F.R.G.		4.6	1.4-12.7		3127	1979	62, 97	AAS
Duisberg, F.R.G.[3]	4.53				690	1979	62, 97	AAS
Gummersbach, F.R.G.[3]	2.74				40	1979	62, 97	AAS

Table 5.5 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Uvdal, Norway	1.22		0.22-6.32	1.23	79	1200-1804	73	"Boat"-AAS
Stolberg, F.R.G.	7.1	5.9	1.5-38.5	0.85	302	1982	74, 75	GFA-AAS
Gummersbach, F.R.G.	4.2	3.8	1.6-9.4	0.70	85	1982	74, 75	GFA-AAS
Stolberg, F.R.G.		6.0	1.5-38.5		317	1982	74, 75	GFA-AAS
Newcastle on Tyne, U.K.	4.42		1.32-7.36	1.60	23	1978	78	ASV
Charlestown, South Carolina, USA.	92.4		33.0-167.9	41.9	18	1974	92	AAS
Tokyo, Japan			6.20-8.24			1973	94	AAS
Toyama, Japan			6.20-8.24			1973	94	AAS

Notes: (1) Ash weight values given, (Ash weight=dry weight-21$\pm$3%).

(2) log standard deviation on $\log_{10}$ transformed data.

(3) Chemical Abstract only.

(4) Unless otherwise specified, AAS is assumed to be flame atomisation.

Table 5.5 cont.

Notes: (5) S.D. means standard deviation.

(6) No. is the size of the sample in the study.

(7) Year of publication, unless date of collection or date of tooth known.

(8) BDL means below detection limit.

Table 5.6

Levels of Lead in Various Parts of Deciduous Teeth (in $\mu gPbg^{-1}$ dry weight).

Part A: Surface Enamel

Location	Mean	Median	Range	S.D.	Depth (μm)	No.	Year	Reference	Analytical Method
USA.			31-500		100-200	4	1972	24	ASV
USA.	BDL					14	1972	36	EIXE
Belgium	≈1600		250-10 250		1	180	1981	79	PIXE
Sweden			300-2000		0.1	2	1978	89	SIMS
Sweden			90-200		1	2	1978	89	SIMS

Part B: Enamel

Location	Mean	Median	Range	S.D.	Depth (μm)	No.	Year	Reference	Analytical Method
Parowvallei, S.A.	2919			2081		27	1982	13	AAS

Table 5.6 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Bothasig, S.A.	10 952			3714	21	1982	13	AAS
Pennsylvania, USA.	33.3		11-52	14.2	11	1972	24	ASV
Severnside, U.K.	30.5		28-32	1.7	4	1972-73	25	AAS
USA.	BDL				14	1972	36	EIXE
South Australia	N.S.				15	1981	37	PIXE
Norway	1.72				56	1978	59	"Boat"-AAS
Japan	0.157					1979	60	GFA-AAS
Helsinki, Finland	69.9			68.4	68	1974	61	AAS
Tervola, Finland	81.4			44.6	68	1974	61	AAS
Sweden			70-200		4	1978	89	SIMS

Table 5.6 cont.

Part C: Dentine.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Chelsea, Somerville, Mass., USA.		12	1-40		3329	1975-78	6, 43, 44	ASV
Bothasig, S.A.	22 733			4423	21	1982	13	AAS
Parowvallei, S.A.	19 926			8296	27	1982	13	AAS
Christchurch, N.Z. (old housing)	6.9	5.7	1.4-27.1	4.6	144	1980	15	GFA-AAS
Christchurch, N.Z. (housing 1940+)	5.6	4.8	1.4-25.0	5.6	145	1980	15	GFA-AAS
Hopi Indians, USA.	7.0			3.8	10	1600-1700	22, 82	XF
Hopi Indians, USA.	27.6			15.2	16	1977	22, 82	XF
California, USA.	16.6			5.4	12	1977	22, 82	XF
Pennsylvania, USA.	27			12	7	1972	24	ASV
Severnside, U.K.	24			4	4	1976	25	AAS

Table 5.6 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
USA.	N.S.				14	1972	36	EIXE
South Australia	N.S.				15	1981	37	PIXE
"Lead poisoned" USA.	54.8			10.8	9	1973	45	ASV
Suburban Boston, USA.	16.9			2.7	20	1973	45	ASV
Iceland	5.4			0.6	17	1973	45	ASV
Norway	4.07				56	1978	59	"Boat"-AAS
Japan	0.104					1979	60	GFA-AAS
Helsinki, Finland	0.71			0.82	68	1974	61	AAS
Tervola, Finland	0.46			0.25	68	1974	61	AAS
Sweden			20-100		4	1978	89	SIMS

Table 5.6 cont.

Part D: Circumpulpal Dentine

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Pennsylvania, USA.	188		69-379	134	7	1972	24	ASV
Richmond, Virginia, USA. "Lead exposed".	194.5			31.8	32	1975	35	ASV
Richmond, Virginia, USA. "Control group".	103.2			12.5	36	1975	35	ASV
USA.	Higher than rest of tooth.				14	1972	36	EIXE
South Australia	N.S.				15	1981	37	PIXE
Philadelphia, USA. Zone 5.	43		0-200		379	1974	40, 41, 42	ASV
Philadelphia, USA. Zone 8.	161		0-400		282	1974	40, 41, 42	ASV
"Lead poisoned" USA.	606.8			61.6	9	1973	45	ASV
Suburban Boston, USA.	84.4			12.7	20	1973	45	ASV
Iceland	35.5			7.4	17	1973	45	ASV

Table 5.6 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Norway			100-500		4	1978	89	SIMS

Notes: (1) Year of publication, unless date of collection or date of tooth known.

(2) Unless otherwise specified, AAS is assumed to be flame atomisation.

(3) S.D. means standard deviation.

(4) No. is the size of the sample in the study.

(5) N.S. means not stated.

(6) BDL means below detection limit.

Table 5.7

Comparison of Lead Levels in Various Parts of Deciduous Teeth (in $\mu gPbg^{-1}$ dry weight).

Location	Surface Enamel	Bulk Enamel	A.D.J.	Dentine	Circumpulpal Dentine	No.	Year	Ref.	Analytical Method
Bothasig, S.A.		10 952±3714		22 733±4423		21	1982	13	AAS
Parowvallei, S.A.		2919±2081		19 926±8296		27	1982	13	AAS
Pennsylvania, USA.	235±231	25±19				4	1972	24	ASV
Pennsylvania, USA.		38±9		27±12	188±134	7	1972	24	ASV
U.K.		30.5±1.7		23.8±4.0		4	1976	25	AAS
"Lead poisoned" USA.				54.8±10.8	606.8±61.6	9	1973	45	ASV
Suburban Boston, USA.				16.9±2.7	84.4±12.7	20	1973	45	ASV
Iceland				5.4±0.6	35.5±7.4	17	1973	45	ASV
Norway		1.72		4.07		56	1978	59	"Boat"-AAS
Japan[1]		0.157		0.104			1979	60	GFA-AAS
Helsinki, Finland		69.9±68.4		0.71±0.82		78	1974	61	AAS
Tervola, Finland		81.4±44.6		0.46±0.25		78	1974	61	AAS

Table 5.7 cont.

Location	Surface Enamel	Bulk Enamel	A.D.J.	Dentine	Circumpulpal Dentine	No.	Year	Ref.	Analytical Method
Sweden. (High fluoride)	≈700	≈90	≈75	≈80	≈140	1	1978	89	SIMS
Sweden, (Low fluoride)	≈300	≈90	≈30	≈150	≈250	1	1978	89	SIMS

Notes: (1) Chemical Abstract only obtained.

(2) Year of publication, unless date of collection or date of tooth known.

(3) Unless otherwise specified, AAS is assumed to be flame atomisation.

(4) No. is the size of the sample in the study.

(5) Ref. means reference.

(6) A.D.J. means amelo-dentine junction.

(7) Values in the table are mean±standard deviation.

Table 5.8

Lead Levels in Various Types of Deciduous Teeth (in $\mu gPbg^{-1}$ dry weight).

Part A: Incisors

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
Cleveland, USA. Urban	55.7			20.5	3911	1978	8	AAS
Cleveland, USA. Suburban.	54.5				2852	1978	8	AAS
St. Louis, USA. "Lead paint zone".		17.8			54	1975	9	GFA-AAS
St. Louis, USA. "Public housing zone"		19.9			22	1975	9	AAS
St. Louis, USA. City (no lead paint).		10.1			48	1975	9	AAS
St. Louis, USA. Suburban.		11.0			19	1975	9	AAS

Table 5.8 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
St. Louis, USA. Industrialised.		10.8			27	1975	9	AAS
Tennessee, USA.	5.2			6.9	85	1975	10	AAS
Birmingham, U.K. 1st incisor.	21.3	19.6		8.28	20	1977	16, 95	AAS
Birmingham, U.K. 2nd incisor.	19.2	17.1		9.75	45	1977	16, 95	AAS
New York, USA[1]			5-60		96	1974	53	AAS
Duisberg, F.R.G.	4.53		1.4-12.7		690	1979	62, 97[2]	AAS
Gummersbach, F.R.G.	4.2	3.8	1.6-9.4	0.70	85	1982	74	GFA-AAS
Stolberg, F.R.G.	7.1	5.9	1.5-38.5	0.85	302	1982	74, 75	GFA-AAS
Newcastle on Tyne, U.K	5.42		2.06-7.50	2.22	5	1978	78	ASV
Gummersbach, F.R.G[2]	2.75				40	1979	97	AAS

Table 5.8 cont.

Part B: Canines

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
St. Louis, USA. "Lead paint zone".	16.8				23	1975	9	GFA-AAS
Tennessee, USA.	5.0			36	19	1975	10	AAS
Birmingham, U.K.	12.7	11.7		5.69	558	1977	16, 95	AAS
New York, USA[1]			4-18		20	1974	53	AAS
Newcastle on Tyne, U.K.	4.34		2.47-6.29	1.34	7	1978	78	ASV

Part C: Molars

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
St. Louis, USA. "Lead paint zone".	10.8				38	1975	9	AAS

Table 5.8 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
St. Louis, USA. "Public project zone".	9.8				7	1975	9	AAS
St. Louis, USA. City, (lead paint free zone).	5.1				24	1975	9	AAS
St. Louis, USA. Suburban.	7.9				9	1975	9	AAS
St. Louis, USA. Industrialised.	6.2				15	1975	9	AAS
Tennessee, USA.	4.7			4.5	49	1975	10	AAS
Glasgow, Scotland. (New housing).	8.4			5.5	39	1978	12	Polarograph
Glasgow, Scotland. (housing with lead pipe)	20.3			8.6	19	1978	12	Polarograph
Birmingham, U.K. 1st Molar	11.3	10.1		7.53	447	1977	16, 95	AAS
Birmingham, U.K. 2nd Molar	9.2	7.8		6.11	322	1977	16, 95	AAS

Table 5.8 cont.

Location	Mean	Median	Range	S.D.	No.	Year	Reference	Analytical Method
New York, USA[1]			2-60		510	1974	53	AAS

Notes: (1) Ash weight, not dry weight (Ash weight = dry weight - 21±3%)

(2) Chemical Abstract only obtained.

(3) No. is the size of the sample in the study.

(4) Unless otherwise specified, AAS is assumed to be flame atomisation.

(5) S.D. means standard deviation.

(6) Year of publication, unless date of collection or date of tooth known.

Table 5.9

Comparison of Lead Levels in Various Types of Deciduous Teeth in Different Teeth Zones.

Whole Teeth

Location	Incisors	Canines	Molars	Year	Ref.	Analytical Method
St. Louis, USA. (Lead paint zone).	17.8 (54)	16.8 (23)	10.8 (38)	1975	9	AAS
St. Louis, USA. (Public housing zone).	19.9 (22)		9.8 (7)	1975	9	AAS
St. Louis, USA. City (No lead paint).	10.1 (48)		5.1 (24)	1975	9	AAS
St. Louis, USA. Suburban	11.0 (19)		7.9 (9)	1975	9	AAS
St. Louis, USA. Industrialised	10.8 (27)		6.2 (15)	1975	9	AAS
Tennessee, USA.	5.2±6.9 (85)	5.0±3.6 (19)	4.7±4.5 (49)	1975	10	AAS
Birmingham, U.K.	20.3±9.02(65)	12.7±.69 (558)	10.3±6.83 (769)	1977	16, 95	AAS

Table 5.9 cont.

Location	Incisors	Canines	Molars	Year	Ref.	Analytical Method
New York, USA.[3]	5-60 (96)	4-18 (20)	2-60 (510)	1974	53	AAS
Newcastle on Tyne, U.K	5.42±2.22 (5)	4.34±1.34 (7)	4.39±1.61 (21)	1978	78	ASV

Enamel

Location	Incisors	Canines	Molars	Year	Ref.	Analytical Method
Pennsylvania, USA.	39±15 (3)		37±5 (4)	1972	24	ASV
Pennsylvania, USA.		37±22 (2)	13±3 (2)	1972	24	ASV

Table 5.9 cont.

Dentine

Location	Incisors	Canines	Molars	Year	Ref.	Analytical Method
Christchurch, N.Z. (High lead (set))	8.1±4.8 (84)	8.5±5.7 (31)	8.1±6.5 (29)	1980	15	GFA-AAS
Christchurch, N.Z. (Low lead (set))	5.6±2.2 (76)	7.1±4.9 (31)	4.1±2.6 (38)	1980	15	GFA-AAS
Pennsylvania, USA.	24±3 (3)		29±16 (4)	1972	24	ASV
Philadelphia and Boston, USA. (Lead poisoned).	122±27 (2)	106±21 (2)	216±16 (2)	1973	45	ASV

Notes: (1) Values are mean±standard deviation.

(2) Number in brackets is the number in the sample.

(3) Ash weight, not dry weight (Ash weight = dry weight -21±3%).

References.

1. Curzon, M.E.J; Bibby, B.G. Effect of heavy metals on dental caries and tooth eruption. J. Dent. Child., 37, 463-465, (1970).

2. Curzon, M.E.J; Crocker, D.C; Font, G.P; Foster, S.P; Jacobs, R.A. Dental caries related to trace element composition of whole human enamel. U.S. NTIS PB Rep. 1976, PB-269957, pp 118.

3. Curzon, M.E.J; Losee, F.L; Dental caries and trace element composition of whole human enamel: Eastern United States. J. Am. Dent. Assoc., 94, 1146-1150, (1977).

4. Curzon, M.E.J; Spector, P.C; Losee, F.L; Crocker, D.C. Dental caries related to Cd and Pb in whole human dental enamel. Trace Sub. Environ. Health, 11, 23-29, (1977).

5. Curzon, M.E.J; Crocker, D.C. Relationships of trace elements in human tooth enamel to dental caries. Arch. Oral Biol., 23, 647-653, (1978).

6. Needleman, H.L; Gunnoe, C; Leviton, A; Reed, R; Peresie, H; Maher, C; Barrett, P. Defecits in psychologic and classroom performance of children with elevated dentine lead levels. N. Engl. J. Med., 300, 689-695, (1979).

7. Annegarn, H.J; Jodaikin, A; Cleaton-Jones, P.E; Sellschop, J.P.F; Madiba, C.C.P; Bibby, D. PIXE analysis of caries related trace elements in tooth enamel. Nucl. Instr. Methods, 181, 323-326, (1981).

8. Kelsall, M.A; Hunter, R.E. Biological indicator of summational exposures to lead. Tooth lead in children

living in Cleveland and its suburbs. Report 1978, EPA/600/1-78/053, Order No. PB-286196, pp 173.

9. Lockeretz, W. Lead content of deciduous teeth of children in different environments. Arch. Environ. Health, 30, 583-587, (1975).

10. Chatman, T; Wilson, D.J. Lead levels in human deciduous teeth in Tennessee. Environ. Lett., 8, 173-183, (1975).

11. Delves, H.T; Clayton, B.E; Carmichael, A; Bubear, M; Smith, M. An appraisal of the analytical significance of tooth-lead measurements as possible indices of environmental exposure of children to lead. Ann. Clin. Biochem., 19, 329-337, (1982).

12. Moore, M.R; Campbell, B.C; Meredith, P.A; Beattie, A.D; Goldberg, A; Campbell, D. The association between lead concentrations in teeth and domestic water lead concentrations. Clin. Chim. Acta, 87, 77-83, (1978).

13. van Wyk, C.W; Grobler, S.R. Lead levels in the teeth of children from selected areas in the Cape Peninsula Letter. S.A. Med. J., 62, 230, (1982).

14. Oehme, M; Lund, W; Jonsen, J. The determination of copper, lead, cadmium and zinc in human teeth by anodic stripping voltammetry. Anal. Chim. Acta, 100, 389-398, (1978).

15. Fergusson, J.E; Jansen, M.L; Sheat, A.W. Lead in deciduous teeth in relation to environmental lead. Environ. Technol. Lett., 1, 376-383, (1980).

16. Mackie, A.C; Stephens, R; Townshend, A; Waldron, H.A. Tooth lead levels in Birmingham children. Arch. Environ. Health, 32, 178-185, (1977).

17. Attramadal, A; Jonsen, J. The content of lead, cadmium, zinc and copper in deciduous and permanent human teeth. Acta. Odontol. Scand, 34, 127-131, (1976).

18. Attramadal, A; Jonsen, J. Heavy trace elements in ancient Norwegian teeth. Acta Odontol. Scand, 36, 97-101, (1978).

19. Little, M.F; Steadman, L.T. Chemical and physical properties of altered and sound enamel - IV. Trace element composition. Arch. Oral Biol., 11, 273-278, (1966).

20. Altshuller, L.F; Halak, D.B; Landing, B.H; Kehoe, R.A. Deciduous teeth as an index of body burden of lead. J. Pediatr., 60, 224-229, (1962).

21. Hardwick, J.L; Martin, C.J. A pilot study using mass spectrometry for the estimation of the trace element content of dental tissues. Helv. Odont. Acta, 11, 62-70, (1967).

22. Kuhnlein, H.V. Calloway, D.H. Minerals in human teeth: differences between preindustrial and contempory Hopi Indians. Am. J. Clin. Nutr., 30, 883-886, (1977).

23. Needleman, H.L; Tuncay, O.C; Shapiro, I.M. Lead levels in deciduous teeth of urban and suburban American children. Nature, 235, 111-112, (1972).

24. Shapiro, I.M; Needleman, H.L; Tuncay, O.C. The lead content of human deciduous and permanent teeth. Environ. Res., 5, 467-470, (1972).

25. Stack, M.V; Burkitt, A.J; Nickless, G. Trace metals in teeth at birth (1957-1963 and 1972-1973). Bull. Environ. Contam. Toxicol., 16, 764-766, (1976).

26. Stewart, D.J. Teeth as indicators of exposure of children to lead. Arch. Dis. Child., 49, 895-897, (1974).

27. Losee, F; Cutress, T.W; Brown, R. Trace elements in human dental enamel. Trace Sub. Environ. Health-VII 1974, a Symp. D.D. Hemphell. Ed. University of Missouri, Columbia, pp 13-17.

28. Losee, F.L; Curzon, M.E.J; Little, M.F. Trace element concentrations in human enamel. Arch. Oral Biol., 19, 467-470, (1974).

29. Burkitt, A.J; Nickless, G; Stack, M.V. Lead in teeth during the perinatal period. Postgrad. Med. J., 51, 778-779, (1975).

30. Bloch, P; Garavaglia, G; Mitchell, G; Shapiro, I.M. Measurement of lead content of children's teeth in situ by X-ray fluorescence. Phys. Med. Biol., 20, 56-63, (1976).

31. Ahlberg, M; Akselsson, R. Proton-induced X-ray emission in the trace analysis of human tooth enamel and dentine. Int. J. App. Radiat. Isot., 27, 279-290, (1976).

32. Johansson, T.B; Ahlberg, M; Akselsson, R; Johansson, G; Malmqvist, K. Analytical use of proton-induced X-ray emission. J. Radioanal. Chem., 32, 207-217, (1976).

33. Möller, B; Carlsson, L-E; Johansson, G.I; Malmqvist, K.G; Hammarstrom, L; Berlin, M. Lead levels determined in Swedish permanent teeth by particle-induced X-ray emission. Scand. J. Work Environ. Health, 8, 267-272, (1982).

34. Besic, F.C; Bayard, M; Wiemann, Jr. M.R.; Burrell, K.H. Composition and structure of dental enamel: elemental composition and crystalline structure of dental enamel as they relate to its solubility. J. Am. Dent. Assoc., 91, 594-601, (1975).

35. de la Burdé, B; Shapiro, I.M. Dental lead, blood lead, and pica in urban children. Arch. Environ. Health, 30, 281-284, (1975).

36. Carroll, K.G; Needleman, H; Tuncay, O.C; Shapiro, I.M. The distribution of lead in human deciduous teeth. Experientia, 28, 434-435, (1972).

37. Chaudhri, M.A; Crawford, A. The influence of trace elements on fluoride uptake by teeth. Nucl. Instrum. Methods, 181, 327-331, (1981).

38. Cohen, D.D; Clayton, E; Ainsworth, T. Preliminary investigations of trace element concentrations in human teeth. Nucl. Instrum. Methods Phys. Res., 188, 203-209, (1981).

39. Malik, S.R; Fremlin, J.H. A study of lead distribution in human teeth, using charged particle activation analysis. Caries Res., 8, 283-292, (1974).

40. Needleman, H.L; Shapiro, I.M. Dentine lead levels in asymptomatic Philadelphia school children: subclinical exposure in high and low risk groups. Environ. Health Perspect., 7, 27-31, (1974).

41. Needleman, H.L; Davidson, I; Sewell, E.M; Shapiro, I.M. Subclinical lead exposure in Philadelphia school children. Identification by dentine lead analysis. N. Engl. J. Med., 290, 245-248, (1974).

42. Needleman, H.L; Shapiro, I.M. Prevalence of

subclinical lead exposure in 761 asymptomatic school children: dentine lead levels as a retrospective marker. Comm. Eur. communities, (rep.), Eur. 1974 (Pub. 1975). Eur. 5360, Proc. Int. Symp. Recent Adv. Asses. Health Eff. Environ. Pollution, 2, 1155-1169.

43. Needleman, H.L; Gunnoe, C; Leviton, A; Peresie, H. Neuropsychological dysfunction in children with "silent" lead exposure. Am. Pediat. Soc., Soc. Pediat. Res. N.Y., April 27, 1978.

44. Needleman, H.L. Studies in children exposed to low levels of lead. Report 1981, EPA-600/1-81-066. Order No. PB 82-108432 pp 72.

45. Shapiro, I.M.; Dobkin, B; Tuncay, O.C; Needleman, H.L. Lead levels in dentine and circumpulpal dentine of deciduous teeth of normal and lead poisoned children. Clin. Chim. Acta, 46, 119-123, (1973).

46. Shapiro, I.M; Mitchell, G; Davidson, I; Katz, S.H. The lead content of teeth. Evidence establishing new minimal levels of exposure in a living preindustrialized human population. Arch. Environ. Health, 30, 483-486, (1975).

47. Stack, M.V; Burkitt, A.J; Nickless, G. Characterisation of teeth by trace elements. Int. J. Forens. Dent., 2, 62-65, (1974).

48. Wipawin, W.T; Thomas, B.J; Khan, H.A. On the uses of solid state nuclear track detectors for low level α-counting. Int. J. Radiat. Phys. Chem., 6, 203-210, (1974).

49. Ericson, J.E; Shirahata, H; Patterson, C.C. Skeletal concentrations of lead in ancient Peruvians. N. Engl.

J. Med., 300, 946-951, (1979).

50. Grandjean, P; Nielsen, O.V; Shapiro, I.M. Lead retention in ancient Nubian and contemporary populations. J. Environ. Path. Toxicol., 2, 781-787, (1979).

51. Lappalainen, R; Knuuttila, M. The distribution and accumulation of Cd, Zn, Pb, Cu, Co, Ni, Mn and K in human teeth from five different geological areas of Finland. Arch. Oral Biol., 24, 363-368, (1979).

52. Lappalainen, R; Knuuttila, M. The concentrations of Pb, Cu, Co and Ni in extracted permanent teeth related to donors' age and elements in the soil. Acta Odontol. Scand., 39, 163-167, (1981).

53. Albert, R.E; Shore, R.E; Sayers, A.J; Strehlow, C; Kneip, T.J; Pasternack, B.S; Friedhoff, A.J; Covan, F; Cimino, J.A. Follow-up of children overexposed to lead. Environ. Health Perspect., 7, 33-39, (1974).

54. Kaneko, Y; Inamori, I; Nishimura, M. Zinc, lead, copper and cadmium in human teeth from different geographical areas in Japan. Bull. Tokyo Dent. Coll., 15, 233-243, (1974).

55. Steenhout, A; Pourtois, M. Lead accumulation in teeth as a function of age with different exposures. Br. J. Ind. Med., 38, 297-303, (1981).

56. Strehlow, C.D; Kneip, T.J. The distribution of lead and zinc in the human skeleton. Am. Ind. Hyg. Assoc., J., 30, 372-378, (1969).

57. Coles, S; Fletcher, R.P; Stack, M.V, Lead, zinc, cadmium and copper levels in mature and immature teeth-Meeting. J. Dent. Res., 59, (SID) 1845, (1980).

58. Deschamps, N; Verhois, V; Calza, F. Determination of trace elements in tooth enamel using neutron activation analysis. J. Radioanal. Chem., 70, 109-116, (1982).

59. Fosse, G; Justesen, N-P.B. Lead in deciduous teeth of Norwegian children. Arch. Environ. Health, 33, 166-175, (1978).

60. Miyazaki, Takeshi. Quantitative analysis of trace elements in the primary teeth. Shika Igaku, 42, 43-60, (1979).

61. Rytömaa, I; Tuompo, H. Lead levels in deciduous teeth. Naturewissenschaften, 61, 363, (1974).

62. Winneke, G; Hrdina, K-G; Brockhaus, A. Neuropsychological studies in children with elevated tooth-lead concentrations. Part I: Pilot study. Int. Arch. Occup. Environ. Health, 51, 169-183, (1982).

63. Yamamoto, Toshiko H; Oshio, Hideo. Determination of cadmium, zinc and lead in human teeth by atomic absorption spectrophotometry. Koku Eisei Gakkai Zasshi, 23, 192-207, (1973).

64. Khandekar, R.N; Ashawa, S.C; Kelkar, D.N. Dental lead levels in Bombay inhabitants. Sci. Total Environ., 10, 129-133, (1978).

65. Szmytowna, Maria; Wiza-Gierlach, Teresa. Determination of trace elements in the hardened tooth deposits of industrial workers. Ann. Pharm. (Poznan), 6, 109-114, (1967).

66. Mishima, Masao; Hoshiai, Takashi; Watanuki, Tomohiko; Sugawara, Kazuo. Ion-exchange and atomic absorption spectrometric determination of trace metals in human

teeth. Koshu Eisiin Kenkyu Hokoku, 30, 40-45, (1981).

67. Brudevold, F; Steadman, L.T. The distribution of lead in human enamel. J. Dent. Res., 35, 430-437, (1956).

68. Dmitriev, I.B. Use of chemical composition of tooth enamel in forensic medicine. Sb. Tr. Sud. Med. Sud. Khim., No. 3. 393-397, (1969).

69. Lerner, G.R. Spectrographic determination of trace elements in bone and hard tooth tissues with halogenation of the samples in the electrode. Ter. Stomatol., No. 7, 59-62, (1973).

70. Steadman, L.T; Brudevold, F; Smith, F.A; Gardner, D.E; Little, M.F. Trace elements in ancient Indian teeth. J. Dent. Res., 38, 285-292, (1959).

71. Langmyhr, F.J; Sundli, A; Jonsen, J. Atomic absorption spectrometric determination of cadmium and lead in dental material by atomization directly from the solid state. Anal. Chim. Acta, 73, 81-85, (1974).

72. Wilkinson, D.R; Palmer, W. Lead in teeth as a function of age. Am. Lab., 7, 67-70, (1975).

73. Fosse, G; Wesenberg, G.B.R. Lead cadmium, zinc and copper in deciduous teeth of Norwegian children in the pre-industrial age. Int. J. Environ. Stud., 16, 163-170, (1981).

74. Ewers, U; Brockhaus, A; Winneke, G; Freier, I; Jermann, E; Krämer, U. Lead in deciduous teeth of children living in a non-ferrous smelter area and a rural area of the FRG. Int. Arch. Occup. Environ. Health, 50, 139-151, (1982).

75. Winneke, G; Kramer, U; Brockhaus, A; Ewers, U;

Kujanek, G; Lechner, H; Janke, W. Neuropsychological studies in children with elevated tooth lead concentrations. Part II: Extended study. Int. Arch. Occup. Environ. Health, 51, 231-252, (1983).

76. Brudevold, F; Reda, A; Aasenden, R; Bakhos, Y. Determination of trace elements in surface enamel of human teeth by a new biopsy procedure. Arch. Oral Biol., 20, 667-673, (1975).

77. Brudevold, F; Aasenden, R; Srinivasian, B.N; Bakhos, Y. Lead in enamel and saliva, dental caries and the use of enamel biopsies for measuring past exposure to lead. J. Dent. Res., 56, 1165-1171, (1977).

78. Pinchin, M.J; Newham, J; Thompson, R.P.J. Lead, copper and cadmium in teeth of normal and mentally retarded children. Clin. Chim. Acta, 85, 89-94, (1978).

79. Bodart, F; Deconninck, G; Martin, M. Th. Large scale study of tooth enamel. IEEE. Trans. Nucl. Sci., NS-28, 1401-1403, (1981).

80. Lindh, U; Tveit, A.B. Proton microprobe determination of fluorine depth distributions and surface multielement characterization in dental enamel. J. Radioanal. Chem., 59, 167-191, (1980).

81. Shapiro, I.M; Burke, A; Mitchell, G; Bloch, P. X-ray fluorescence analysis of lead in teeth of urban children in situ: correlation between the tooth lead level and the concentration of blood lead and free Erythroporphyrins. Environ. Res., 17, 46-52, (1978).

82. Kühnlein, H.V. Strontium and lead in the Hopi nutritional environment and teeth. Thesis, pp 199, (1976).

83. Al-Naimi, T; Edmonds, M.I; Fremlin, J.H. The distribution of lead in human teeth, using charged particle activation analysis. Phys. Med. Biol., 25, 719-726, (1980).

84. Fremlin, J.H; Tanti-Wipawin, W. Distribution of lead in teeth. Proc, Analyt. Div. Chem. Soc., 13, 195-7, (1976).

85. Fremlin, J.H; Edmonds, M.I. The determination of lead in human teeth. Nucl. Instrum. Methods, 173, 211-215, (1980).

86. Cutress, T.W. A preliminary study of the microelement composition of the outer layer of dental enamel. Caries Res., 13, 73-79, (1979).

87. Galle, P. Biomedical applications of secondary ion emission microanalysis. Springer Ser. Chem. Phys., 9, (Second. ion mass spectrom., SIMS 2), 238-243, (1979).

88. Lodding, A; Odelius, H. In-depth distributions of elements in teeth, determined by ion probe analysis. Micros. Acta Suppl. 2, (Microprobe Anal. Biol. Med.), 367-376, (1978).

89. Petersson, L.G; Lodding, A; Koch, G. Elemental microanalysis of enamel and dentin by secondary ion mass spectrometry (SIMS). Deciduous and permanent teeth from high and low fluoride areas. Swed. Dent. J., 2, 41-54, (1978).

90. Needleman, H.L; Shapiro, I.M. Lead in deciduous teeth. Marker of exposure in heretofore asymptomatic children. Proc., Int. Symp. Environ. Health Aspects Lead 1972, (Pub. 1973), 773-780, Comm. Eur.

Communities, Cent. Inform. Doc.: Luxembourg, Luxembourg.

91. Derise, N.L; Ritchey, S.J. Mineral composition of normal human enamel and dentin and the relation of composition to dental caries: II. microminerals. J. Dent. Res., 53, 853-858, (1974).

92. Habercam, J.W; Keil, J.E; Reigart, J.R; Croft, H.W. Lead content of human blood, hair, and deciduous teeth: correlation with environmental factors and growth. J. Dent. Res., 53, 1160-1163, (1974).

93. Kaneko, Y. Trace metals in human teeth. II. Contents of lead, copper, cadmium and zinc in human teeth. Koku Eisei Gakkai Zasshi, 22, 79-97, (1972).

94. Oshio, Hideo. Contents of cadmium, zinc and lead in deciduous teeth obtained from two different areas in Japan. Koku Eisei Gakkai Zasshi, 23, 208-222, (1973).

95. Stephens, R; Waldron, H.A. Body burdens of lead in Birmingham. J. R. Soc. Health, 96, 176-179, (1976).

96. Bloch, P; Mitchell, G; Shapiro, I.M; Garavaglia, G; Child lead exposure determined from measurement of X-ray fluorescence of teeth in situ. IEEE Trans. Nucl. Sci., NS-24, 577-580, (1977).

97. Ewers, U; Brockhaus, A; Genter, E; Idel, H; Schuermann, E.A. Tooth lead concentrations in school children from different environments of north western Germany. Int. Arch. Occup. Environ. Health, 44, 65-80, (1979).

98. Stack, M.V; Burkitt, A.J; Nickless, G. Lead in children's teeth. Nature, 255, 169, (1975).

99. Aston, E.R. Dental study of employees of five lead plants. Ind. Med. Surg., 21, 17-20, (1952).

Chapter 6

An Investigation of Some of the Factors Which Affect Lead Concentrations in Teeth

6.1.1 Introduction

If teeth are to be used as bio-indicators of a person's past lead exposure, then it is necessary to have a good understanding of the way lead is deposited in teeth. But of even greater importance is a knowledge of the distribution of lead in teeth. As indicated in Chapter 5, the factors found to affect lead concentration in teeth samples are; lead exposure of donor, donor's age, zone of tooth analysed, and the type of tooth.

The work reported in this chapter has been divided into three topic areas:

(a) An investigation of the variation of lead concentration within a particular tooth, with an emphasis on the variation within enamel, and between coronal and root primary dentine. Levels of lead in circumpulpal dentine have also been studied as well as concentration/depth profiles in surface enamel for lead, cadmium, copper, iron, and zinc. These studies were carried out on permanent teeth.

(b) An investigation into the variance of lead concentration with tooth type. This was carried out by the analysis for lead in sixteen partial or complete sets of human permanent teeth.

(c) A follow-up of the work of Fergusson et al. (1), by comparing the results they obtained with levels found in dentine from deciduous teeth of children living in rural Canterbury, New Zealand.

6.1.2 Tooth Morphology.

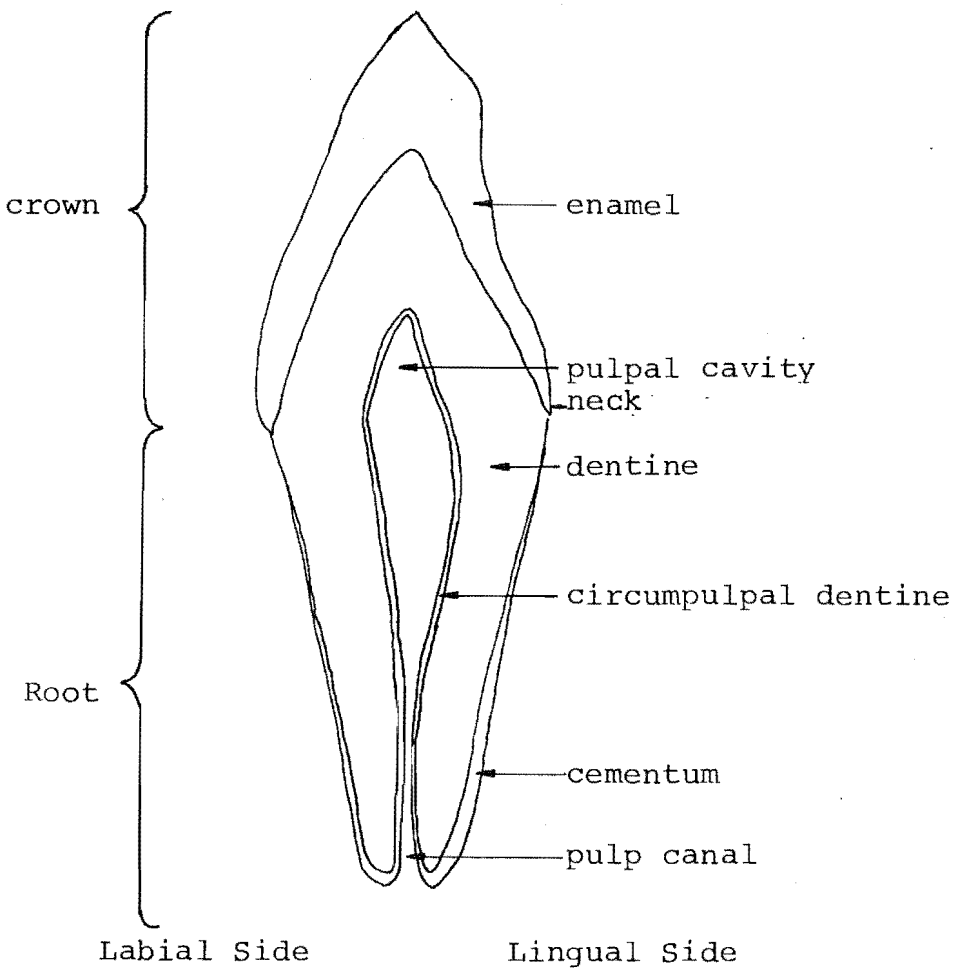
In Figure 6.1, is a diagrammatic representation of a longitudinal section through a lower central incisor. Teeth are made up of two main zones of inorganic material. The first of these is the enamel. The enamel consists of tightly packed hexagonal crystals of apatite prisms, approximately 50-60 nm wide and 25-30 nm thick. Enamel is formed by the calcification of an organic matrix, which is laid down by ameloblasts. As calcification occurs the organic matrix is withdrawn, so that in fully formed enamel the percentage of organic matter is less than 0.8%. The formation of tooth enamel is complete before eruption of the tooth. Enamel is highly impermeable, although it can absorb ions from fluids in which it is in contact.

The other zone in teeth is dentine. The dentine, unlike enamel, grows throughout the life of the tooth and can repair itself in cases of injury. Dentine, unlike enamel, has a high organic content, being approximately 20%-23%, and is permeable. The dentine structure consists of dentine matrix interspersed with dentinal tubules which run from the odontablast layer, in contact with the pulp, through to the amelo-dentine junction. These tubules carry fluid, and are used in both the growth and repair of the dentine structure. The dentine in contact with the pulpal cavity is called circumpulpal dentine and has an even higher

Figure 6.1

Diagram of a Longitudinal Section Through a Tooth.

(Lower Lateral Incisor)



organic content, as it includes the cellular membrane responsible for the formation of dentine.

6.2 Analytical Methods

6.2.1 Preparation of Teeth Prior to Analysis.

All teeth, prior to analysis, were soaked overnight in 1% papain, 1% sodium chloride solution. This helped in the removal of remaining organic matter. The teeth were then scrubbed with a stiff nylon brush to remove any clinging material and any tartar. The teeth were then washed in distilled water and dried at 60^{o}C to constant weight, for 24 hours, in a drying box. A small group of teeth was also washed in an ultrasonic bath. The relative washing techniques were compared by means of the surface concentrations of lead on two groups of teeth. For six teeth treated by soaking in papain and scrubbing with nylon brush only, the mean lead concentration for surface enamel was 1040 $\mu gPbg^{-1}$ (range of 500-1950 μgg^{-1}), for nine different teeth, treated by soaking in papain, scrubbing with a nylon brush and then placing in an ultrasonic bath for fifteen minutes, the mean lead concentration in surface enamel was 1030 $\mu gPbg^{-1}$ (range of 230-2400 $\mu gPbg^{-1}$). Though the results are on different groups of teeth and are not expected to be the same, they strongly suggest that there is no significant difference between the two washing techniques.

6.2.2 Preparation of Teeth for Bulk Enamel and Dentine Studies.

Permanent teeth were used in the study of the variation of lead concentrations in dentine and enamel and in the comparison of dentine and enamel lead levels. A slice, from a cleaned tooth, approximately 1 mm thick, was cut with a diamond saw, along the length of the tooth and from the lingual (tongue facing) to the labial (lip facing), sides of the tooth. The slice was broken into several chips using a steel-carbide chisel. This normally gave four enamel parts, and five or six dentine chips. The circumpulpal dentine and the cementum surfaces were chipped off the dentine chips so that the sample was just primary dentine.

Circumpulpal dentine was removed from the pulpal cavity and collected for some teeth so that comparisons between primary and circumpulpal dentine could be made. A dentist burr drill was used to detach loosely bound circumpulpal dentine from the walls of the pulpal cavity.

In the study of dentine lead concentration variation with tooth types, two chips of dentine were used from the tooth slice. The chips were obtained from approximately midway from the top of the coronal dentine to the bottom of the root. They were also freed from any cementum and circumpulpal dentine, so that lead in these materials did not affect the comparisons between teeth.

In a separate study a sample of deciduous teeth was obtained from children living in rural Canterbury, New Zealand. As the aim was to compare lead levels of the sample with those of Fergusson et al. (1) who measured dentine levels in deciduous teeth of children living in two zones of Christchurch, the teeth were prepared by the same method as Fergusson et al. (1). The teeth were cleaned as outlined

in Section 6.2.1, then a 1 mm longitudinal slice was cut from each tooth, and two chips of dentine, free from enamel, but predominantly root dentine, were chipped with a chisel from the slice.

The digestion of dentine and enamel samples was carried out by treating weighed samples with 0.1ml of concentrated nitric acid. The sample and acid were heated over a hot plate for approximately 10 minutes, or until the solution was clear, during which the sample completely dissolved. After cooling, the solution was made up to 1mL with distilled water.

6.2.3 Preparation of Teeth for Surface Enamel Studies.

To investigate the concentrations of lead, copper, cadmium, iron and zinc in surface enamel, a method was developed similar to that of Brudevold et al. (2, 3). In this method an adhesive backed mask, with a hole of known size was placed on top of the enamel surface. Then 10µL of an acid etching solution consisting of 1.6M hydrochloric acid in 70%V/V glycerol was placed on the exposed enamel surface for a fixed period of time. The etching solution was then washed off with distilled water into 1mL volumetric flasks containing 0.1mL of concentrated nitric acid, and made up to the mark with distilled water. For the determination of calcium and zinc, 0.5mL of this solution was made up to 5mL with 0.5M nitric acid.

A problem with this technique is the choice of mask material. The material initially chosen was an adhesive backed paper tape. This did not stretch to cover the tooth

surface easily and it was fairly quickly attacked by etching acid, making repeated etchings difficult as the acid could seep out under the mask. The second material tried was a PVC insulating tape. While this tape's flexibility made it more suitable as a masking material, the high levels of lead present made it useless for this work. More lead could be extracted from the tape than from the tooth, as lead compounds are added to the PVC to act as a plasticizer. The material finally chosen was an adhesive backed polypropylene film, 60µm in thickness. While this tape is lead free and resistant to acid attack, it is not as flexible as a PVC tape, and care had to be taken with its use, as leakage under the mask could occur.

6.2.4 Preparation of Reagents and Glassware.

All glassware was washed in a detergent solution, washed in distilled water, soaked overnight in approximately 2%W/V EDTA solution, then boiled in this solution for half an hour. The glassware was then washed four times with double distilled water, and then with 2M nitric acid solution which was heated to boiling for one hour. Finally the glassware was washed four more times in redistilled water and dried overnight in a drying box.

The nitric acid originally used was "AR" grade, but was twice distilled in an all glass still to produce an acid with lower blank values than BDH "Aristar" grade. The glycerol used was of "AR" grade but this was then vacuum distilled twice, by which time the blank levels were of similar values to that of the other reagents. The

hydrochloric acid used in the surface etch was of BDH "Aristar" grade. The distilled water from the University of Canterbury's distilled water supply, was redistilled in an all glass still.

6.2.5 Instrument Settings and Checks on Method.

The analysis of enamel and dentine for lead, copper, cadmium, iron and zinc was carried out by graphite furnace atomisation atomic absorption spectrophotometry, (GFA-AAS), using graphite cups. The instruments used were a Varian CRA-63, carbon rod atomiser connected to a Varian AA-1475 atomic absorption spectrophotometer. The output from the instrument was traced onto graph paper, and peak area was used as a measure of the amount of each element present. Instrumental settings are given in Table 6.1.

Calcium was analysed by flame atomisation atomic absorption spectrophotometry using a nitrous oxide/acetylene flame with the Varian AA-1475. This flame was used as phosphate interferes with calcium in the air/acetylene flame but not in the hotter nitrous/acetylene flame (27). Instrument settings for calcium determination are also given in Table 6.1.

As a check on possible contamination during analysis, a sample of powdered primary dentine was used as an internal standard. This "standard" dentine sample was prepared by crushing a large number of adults' teeth and using floatation separation to obtain the dentine fraction. Over 80 determinations were carried out and the lead concentration for this "standard" was found to be $10.3 \mu g Pb g^{-1}$ with an

Table 6.1

Instrument Settings for Analysis of Teeth Material.

Settings for Varian AA-1475	Lead	Cadmium	Copper	Iron	Zinc	Calcium
Wavelength (nm)	217.0	228.3	324.8	248.2	213.9	422.8
Lamp Current (mA)	5	3	3	5	5	4
Band Width (nm)	1.0	0.5	0.5	0.2	1.0	0.2
Mode	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance
Background Correction	No	No	No	No	No	Yes
Atomisation[1]	G.C.	G.C.	G.C.	G.C.	G.C.	C_2H_2/N_2O

Settings for Varian CRA-63	Lead	Cadmium	Copper	Iron	Zinc	Calcium
Dry (Time)	4.5 (10)	4.5 (10)	4.5 (10)	4.5 (10)	4.5 (10)	N/A
Ash (Time)	5.0 (5)	3.5 (5)	5.0(5)	5.0(5)	5.0(5)	N/A
Ramp Rate	3.0	3.0	3.0	3.0	3.0	N/A
Cut Off Volts	7.0	7.0	9.0	9.0	7.0	N/A

Table 6.1 cont.

Settings for Varian CRA-63	Lead	Cadmium	Copper	Iron	Zinc	Calcium
Standards Range ($ngmL^{-1}$)	25-200	1-10	25-200	25-200	0.5-5.0	1-20*

Notes: (1) G.C. means graphite cup.

(2) * means concentration in μgmL^{-1}.

(3) N/A means not applicable.

error in the mean of 0.2μgPbg^{-1}. The range of concentration obtained for this "standard" was 8.7 - 11.9μgPbg^{-1}. This can be compared with the value of 10.3±0.1μgPbg^{-1} obtained by Fergusson et al. (1) with the same standard dentine sample.

A check was also carried out on the "standard" dentine sample by the method of standard additions. The concentration of lead was found to be 10.3±0.7μgPbg^{-1}. This value is not statistically significantly different from that obtained by the use of an absorbance versus concentration calibration curve, therefore a standard calibration curve using aqueous lead standards was used in the analyses.

To check further on the validity of the analytical method chosen, five teeth from the same donor were analysed by anodic stripping voltammetry (ASV) as well as GFA-AAS. The mean and standard deviation for the five samples by ASV was 32.5±.11.5μgPbg^{-1} and the mean and standard deviation for GFA-AAS was 32.4±11.8μgPbg^{-1}. The average percentage difference between ASV and GFA-AAS was 14%. The analysis by ASV had to be done by standard additions as the presence of oxidised "organics" caused interference with the lead potential wave. Considering the possible variation between two samples from the same tooth (see Section 6.3.1) the agreement is satisfactory.

For each analysis of a sample by GFA-AAS, no less than three aliquots of sample were used, as this was normally found to be sufficient to produce consistent peak areas. The following detection limits were used, (although it was possible to measure concentrations below these, the levels in the blanks were the limiting factor), lead 2.0ppb,

cadmium 0.5ppb, copper and iron 5.0ppb and zinc 0.2ppb. These limits refer to aqueous solution.

6.3 Results and Discussion

6.3.1 Lead Levels in Enamel and Dentine.

Previous investigations of lead levels in enamel and dentine of permanent teeth have found that the concentrations in dentine are higher than those found in enamel (4-9). However, several authors have suggested that the ratio of lead concentration in enamel to lead concentration in dentine varies with age, and have found cases of the lead concentration in enamel being higher than in dentine (10-13).

In order to look at the distribution of lead in enamel and dentine, teeth were cut as outlined in Section 6.2.2, the enamel divided into four chips and the dentine divided into four to six chips. A sample of circumpulpal dentine was also analysed for each tooth. The results of these analyses are given in Table 6.2.

The results indicate in all cases but one, that lead concentrations in dentine are higher than lead concentrations in enamel. For the total sample the mean lead concentration in dentine was $20.4\mu gPbg^{-1}$, while the mean lead concentration in enamel was $7.9\mu gPbg^{-1}$. In all cases the concentration of lead was higher in circumpulpal dentine than in primary dentine. The average lead concentration in circumpulpal dentine was $99\mu gPbg^{-1}$, between two and five times higher than primary dentine on an individual tooth basis. Other authors have also found that the circumpulpal dentine has

Table 6.2

Lead Levels in Different Zones of Teeth.

Tooth Type	Enamel	Dentine	Circumpulpal Dentine
Incisor	6.6±0.3	9.5±1.6	
	22.3±5.7	35.6±6.4	68±5
	30.4±11.0	49.4±9.0	134±29
	7.5±2.9	20.3±5.2	112±14
Canine	5.2±1.0	19.1±4.6	88±3
	10.3±3.0	18.9±1.0	81±13
Molar	3.4±0.3	5.2±0.2	
	7.3±0.2	5.3±1.2	
Mean	7.9	20.4	99

Notes: (1) All values for lead concentration is $\mu gPbg^{-1}$ (dry weight).

(2) Values are mean±standard deviation of several determinations on each tooth.

(3) Each row of the table is for one particular tooth.

higher lead concentration than the primary dentine (4, 6, 7, 9, 12-15).

During this study, it was noted that for incisors in particular, the enamel did not appear to have a uniform lead distribution, higher values were found in the enamel near the neck of the tooth, compared with enamel near the tooth's cutting surface. To investigate this further the enamel from the tooth slice of a group of incisors was divided into four regions viz. enamel from the lingual and labial sides and then each side was divided into upper (near the cutting edge) and lower (near the neck) pieces.

The results from these analyses are given in Table 6.3 (a). To enable the grouping of individual results for statistical analysis, the mean lead enamel for each tooth was calculated, then the result for each enamel chip was divided by the mean enamel lead concentration for the tooth from which the chip had been obtained. The mean of these ratios for the lingual side was 1.19 and for the labial side was 0.81. These two values were significantly different at $p<.00001$ level. A similar comparison of the upper and lower chips off each side of the tooth shows a significant difference at the $p<.005$ level (see Table 6.4).

A possible explanation for this difference in lead levels for parts of the enamel is that the lingual surface of the tooth is kept moist by contact with the tongue, while for part of the time the labial surface is relatively drier especially when the mouth is open during exercise and conversation. The greater exposure of the lingual surface to saliva, as witnessed by the greater build up of "tartar" and plaque on this surface, may allow a greater incorporation

Table 6.3 (a)

Lead Concentrations in Different Areas of Enamel from Permanent Teeth (Incisors) in $\mu gPbg^{-1}$ (dry weight).

Tooth Type	Labial Surface		Lingual Surface		Mean
	Upper	Lower	Upper	Lower	
Lower Incisor[1]	6.1(0.86)	4.6(0.65)	9.8(1.39)	7.8(1.10)	7.1
	7.2(0.32)	27.3(1.19)	28.2(1.23)	28.7(1.26)	22.9
	10.3(0.77)	13.3(1.00)	14.9(1.12)	14.8(1.11)	13.3
	18.7(0.73)	27.4(1.07)	22.7(0.89)	33.5(1.31)	25.6
	14.9(0.77)	15.2(0.78)	20.6(1.06)	27.0(1.39)	19.4
	37.4(1.24)	27.3(0.90)		26.1(0.86)	30.3
	14.4(0.56)	30.2(1.17)	27.7(1.07)	31.1(1.20)	25.9
	15.9(0.35)	30.9(0.68)	46.7(1.02)	88.8(1.95)	45.6
	20.2(0.66)	37.5(1.23)	22.6(0.77)	41.4(1.36)	30.4
Lower Incisor[2]	23.9 (0.84)	23.6(0.84)	32.4(1.14)	33.7(1.19)	28.4
	16.5(0.73)	22.1(0.98)	25.7(1.14)	25.8(1.15)	22.5
	13.8(0.51)	25.6(1.06)	17.2(0.71)	40.3(1.66)	24.2

Table 6.3 (a) cont.

Tooth Type	Labial Surface		Lingual Surface		Mean
	Upper	Lower	Upper	Lower	
Upper Incisor[1]	8.2(0.55)	10.1(0.67)	16.8(1.12)	24.9(1.66)	15.0
Upper Incisor[2]	11.4(0.61)	17.2(0.91)	20.2(1.07)	26.4(1.40)	18.8
	17.2(0.70)	26.0(1.06)	29.1(0.82)	35.2(1.43)	24.6
	2.2(0.51)	2.6(0.60)	3.5(0.80)	3.5(0.80)	4.4
	5.1(0.68)	10.5(1.40)	5.0(0.67)	5.0(0.67)	7.5
Mean of Ratios	0.67	0.95	1.00	1.37	
Standard deviation of Ratios	0.21	0.23	0.21	0.31	
Sample Size	17	17	16	17	

	Total Labial	Total Lingual
Mean of Ratios	0.81	1.19
Standard deviation of Ratios	0.26	0.32
Sample Size	34	33

Table 6.3 (b).

Lead Concentrations in Different Areas of Enamel from Permanent Teeth (Molars) in $\mu gPbg^{-1}$ (dry weight).

Tooth Type	Labial Surface		Lingual Surface		Mean
	Upper	Lower	Upper	Lower	
Molar	3.1	3.7	3.5	3.4	3.4
	13.8	23.7	8.5	16.2	15.6
	7.2	7.1	7.5	7.3	7.3

Notes: (1) Central Incisor

(2) Lateral Incisor

(3) Ratio of sample mean given in brackets, (value/mean).

Table 6.4

Students t-Test for Different Enamel Zones.

Zones for Comparison.		Z	p
Labial Upper	: Labial Lower	3.71	<.0005
	: Lingual Upper	4.51	<.0001
	: Lingual Lower	7.75	<.00001
Labial Lower	: Lingual Upper	0.65	N.S.
	: Lingual Lower	4.49	<.0001
Lingual Upper	: Lingual Lower	4.03	<.0005
Total Labial	: Total Lingual	5.33	<.00001

Notes: (1) t-test calculated using the null hypothesis: $H_O: X_1 = X_2$.

(2) N.S. means not significant.

(3) Z is the Z-statistic for the t-distribution and p is the level of significance.

of lead by isomorphous replacement of calcium ions on the lingual surface. In support of this suggestion is the fact that permanent molars do not appear to show this effect. When the enamel from three permanent molars was analysed no discernible pattern was found (see Table 6.3 (b)). The difference between the upper and lower enamel fractions may also be due to the effects of saliva and plaque, as saliva and plaque are found in greater quantities near the neck of the tooth, resting on the gum, giving greater opportunity for tooth surface contact with lead. Another possibility is that lead is incorporated at different rates during tooth growth.

An analysis was also carried out on coronal and root dentine for various types of teeth. Coronal dentine was taken from the area of dentine above the neck of the tooth, and the root dentine from the lower half of the tooth root. In all cases the dentine samples were freed of circumpulpal dentine, cementum and dentine in the vicinity of the amelo-dentine junction. The results of these analyses are presented in Table 6.5. The average coronal dentine for a tooth was then divided by the mean dentine concentration for the same tooth. The mean of these ratios was 0.91. In a similar way the mean of the ratios for root dentine was 1.07. Using the Students t-test, the coronal and root dentine were found to be significantly different at the $p<.005$ level. Stack et al. (16) found roots to have higher levels than crowns, but as the crown is predominantly enamel, and enamel is generally lower in lead concentration than dentine, their results are not strictly comparable with the present findings. Pinchin et al. (17) did find that for deciduous

Table 6.5

Lead Concentrations in Coronal and Root Dentine of Permanent Teeth (in $\mu gPbg^{-1}$ dry weight).

Tooth Type	Dentine		Mean	Ratio (Value/Mean)	
	Root	Coronal		Root	Coronal
Incisor	11.20	8.43	9.5	1.18	0.89
	40.1	30.0	35.6	1.13	0.84
	53.4	43.4	49.4	1.08	0.88
	19.0	21.2	20.3	0.94	1.04
Canine	20.5	17.8	18.9	1.08	0.94
	19.1	19.1	19.1	1.00	1.00
Molar	5.75	5.20	5.3	1.08	0.98
Mean of the ratios				1.07	0.91
Standard deviation of the ratios				0.08	0.09
Number in the sample				7	7

Table 6.5 cont.

Notes: (1) Each row is a result for one tooth.

(2) Using the students t-test for the null hypothesis the two means are the same,

i.e. $H_0: X_1 = X_2$ $Z = 3.52$ $p < .005$

(3) Z is the Z-statistic for the t-distribution and p is the level of significance.

teeth the lead concentration in root tips was higher than the lead concentration in the rest of the dentine. This result is in agreement with the data in Table 6.5 for permanent teeth.

A possible explanation may be that lead can migrate from the pulpal cavity into the dentine, producing a gradient of lead concentration within the dentine. Also as the root dentine is closer to the pulpal cavity lead may diffuse more easily into the root dentine, giving rise to higher lead concentrations in root dentine.

6.3.2 The Analysis of Surface Enamel for Lead, Cadmium, Copper, Iron and Zinc.

Previous studies on surface enamel have shown high levels of lead, (2, 3, 8, 9, 11-14, 19-23), cadmium (20), copper (8, 18, 20-22), iron (8, 13, 18, 20-22) and zinc (2, 8, 13, 18, 20-22) in surface enamel compared with the bulk enamel. To investigate this further, the labial enamel surface of several teeth was analysed by a method similar to Brudevold et al. (2, 3). But in this case, successive etchings were performed at the one spot to get some idea of how rapidly the concentration of these elements falls off with increasing depth into the enamel. However, it is necessary to assume that metals are not mobilised from neighbouring unetched enamel into the etching solution.

The depth of etch was calculated from the calcium concentration in each of the etched solutions, assuming that enamel was 37% calcium by weight and that enamel had a density of $2.95 g cm^{-3}$ (2, 3). The diameter of the mask

was 4.5 mm, giving an area of etch of 15.9 mm^2. The depth values are calculated using the calcium concentration. The values are quoted as half the depth of an individual etch. Hence all depths are the sum of the previous etching depths, plus half the depth of that particular etching.

The data for the concentration of lead, cadmium, copper, iron and zinc in the first etch of the surface enamel from permanent teeth is given in Table 6.6. The values are the average concentration of a number of determinations on different teeth of each element in the first 0.2 to 0.8μm of the enamel surface. Typical depth profiles for lead, copper, iron, and zinc concentrations in dental enamel are given in Figures 6.2-6.5. Results for lead on five separate teeth are given in Table 6.7. No depth profile for cadmium is given as the concentration of cadmium in the surface tooth enamel was so low that the technique used was not sensitive enough to discern cadmium concentrations at lower levels above the background noise levels. The values obtained in this study are in general agreement with values obtained in previous work at similar depths in dental enamel, for all the elements investigated.

The results indicate a significant sorption of the metals onto the surface of the tooth enamel. Since the surface levels are not affected by cleaning methods (see Section 6.2.1), the metals on the surface must be relatively strongly sorbed. Also, since levels are still high in the second and third etched samples it suggests that metal ions are firmly incorporated into the enamel structure. As the enamel consists of a large number of crystals, the depths quoted are only approximations, as the ease of dissolution

Table 6.6

Trace Element Concentrations in Surface Dental Enamel (in $\mu gPbg^{-1}$ dry weight).

Element	Mean	Median	Minimum	Maximum	Number of Different Teeth	Average Sample Depth
Lead	1100	990	500	3400	13	0.52±0.31
Cadmium	7.0	2.3	0.2	23	4	0.45±0.20
Copper	740	370	120	3800	9	0.51±0.17
Iron	850	550	230	3900	9	0.51±0.17
Zinc	1030	790	130	1900	10	0.51±0.16

Notes: (1) The sample depth is in μm, also the value for depth in the table is the mean±standard deviation for the first etch on each tooth.

(2) Depth is half the calculated etch depth, found from the calcium concentration in the etched sample.

Figure 6.2

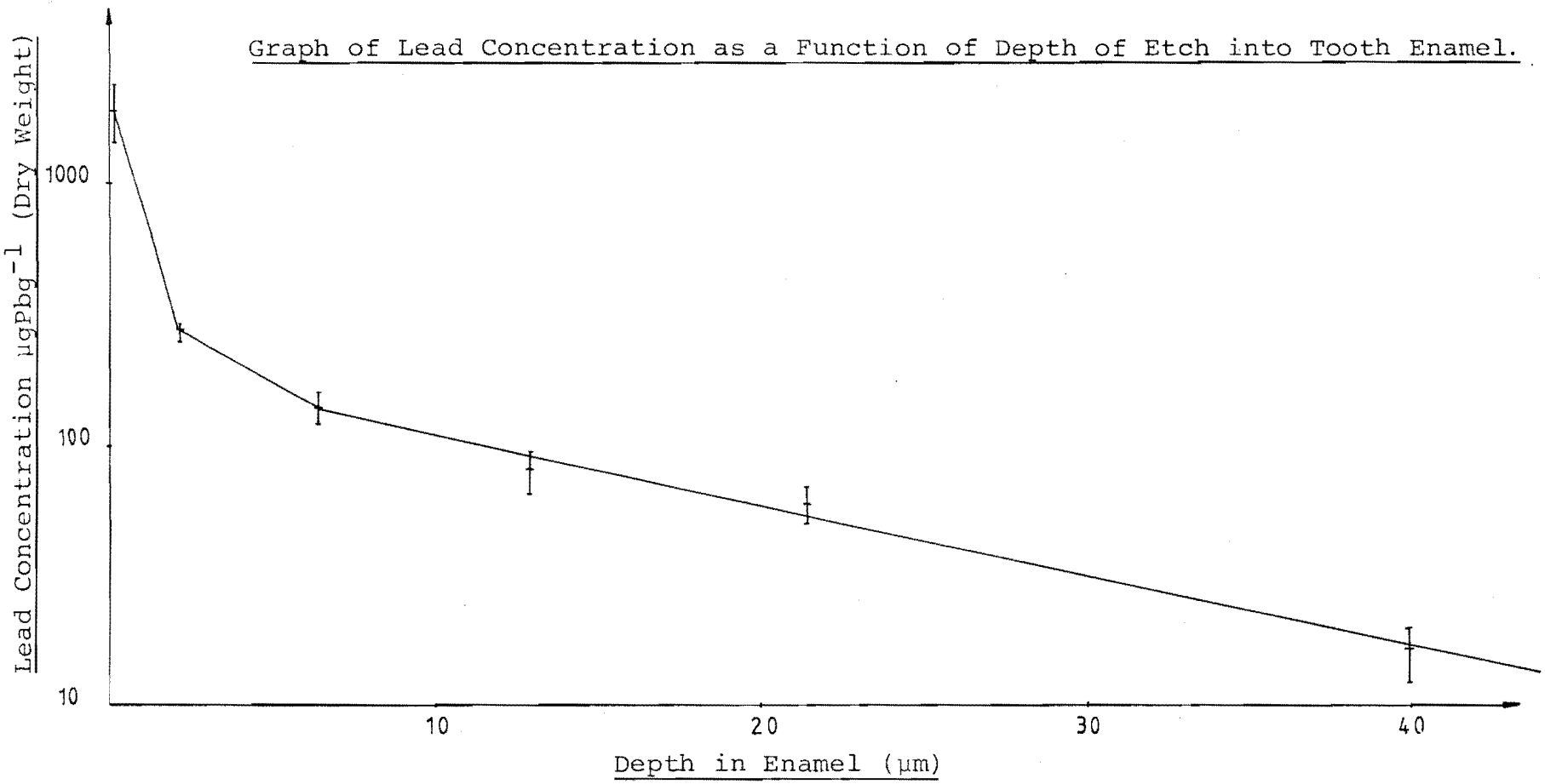

Graph of Lead Concentration as a Function of Depth of Etch into Tooth Enamel.
Lead Concentration μgPbg⁻¹ (Dry Weight)
1000
100
10
10
20
30
40
Depth in Enamel (μm)

Figure 6.3

Graph of Copper Concentration as a Function of Depth of Etch in Enamel.

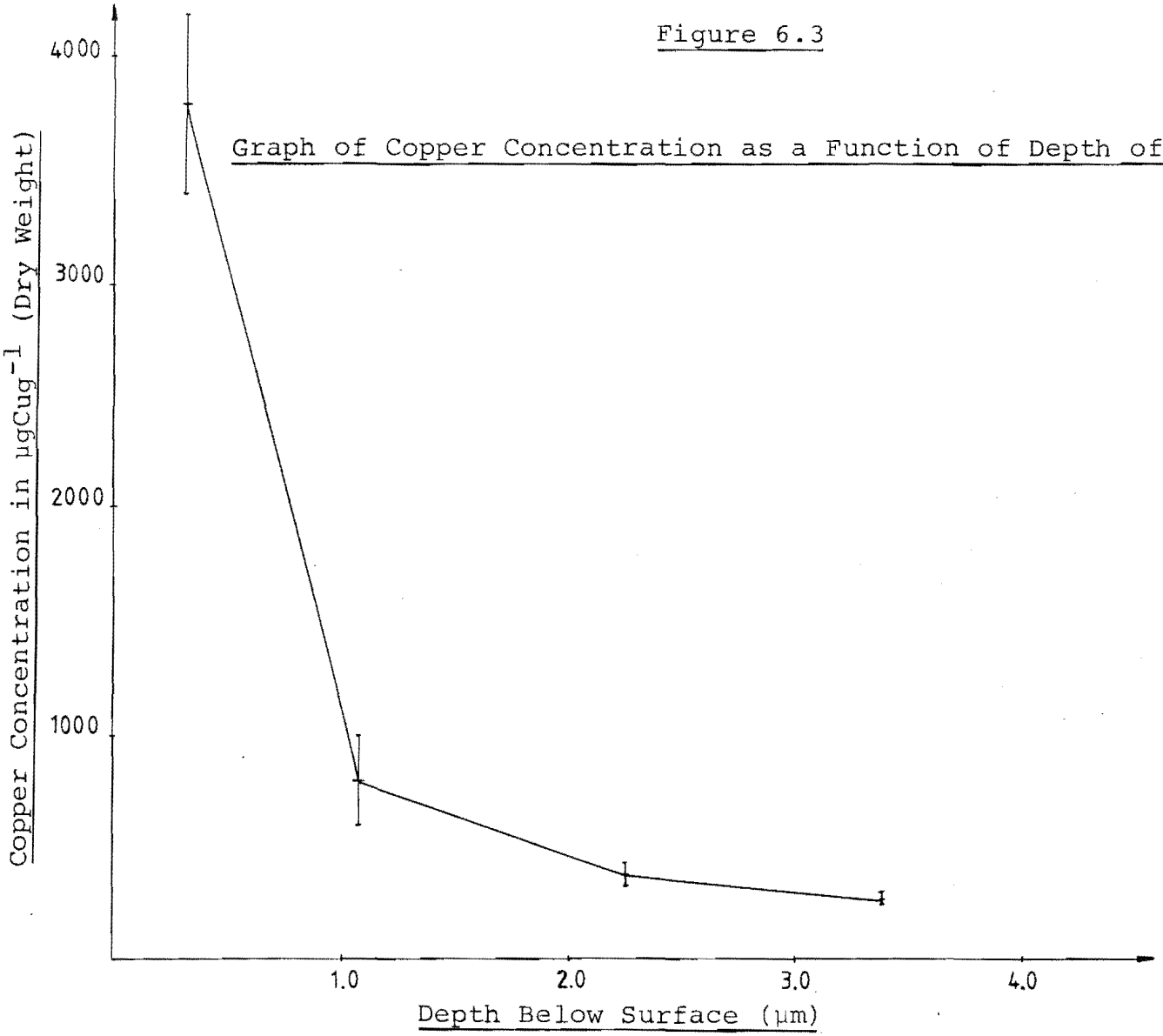


Figure 6.4

Graph of Iron Concentration as a Function of Etch Depth in Enamel,

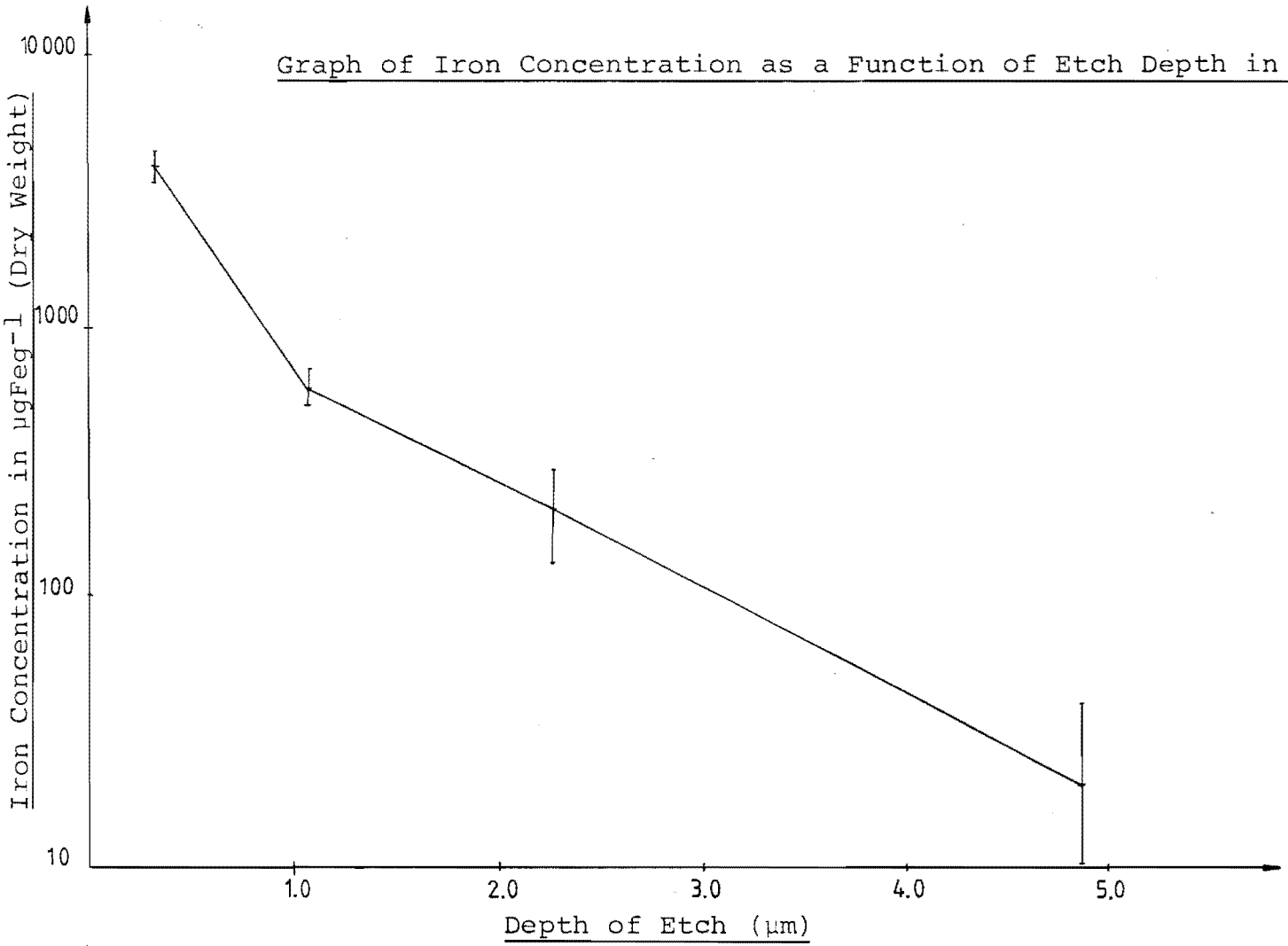


Figure 6.5

Graph of Zinc Concentration as a Function of Depth of Etch in Enamel.

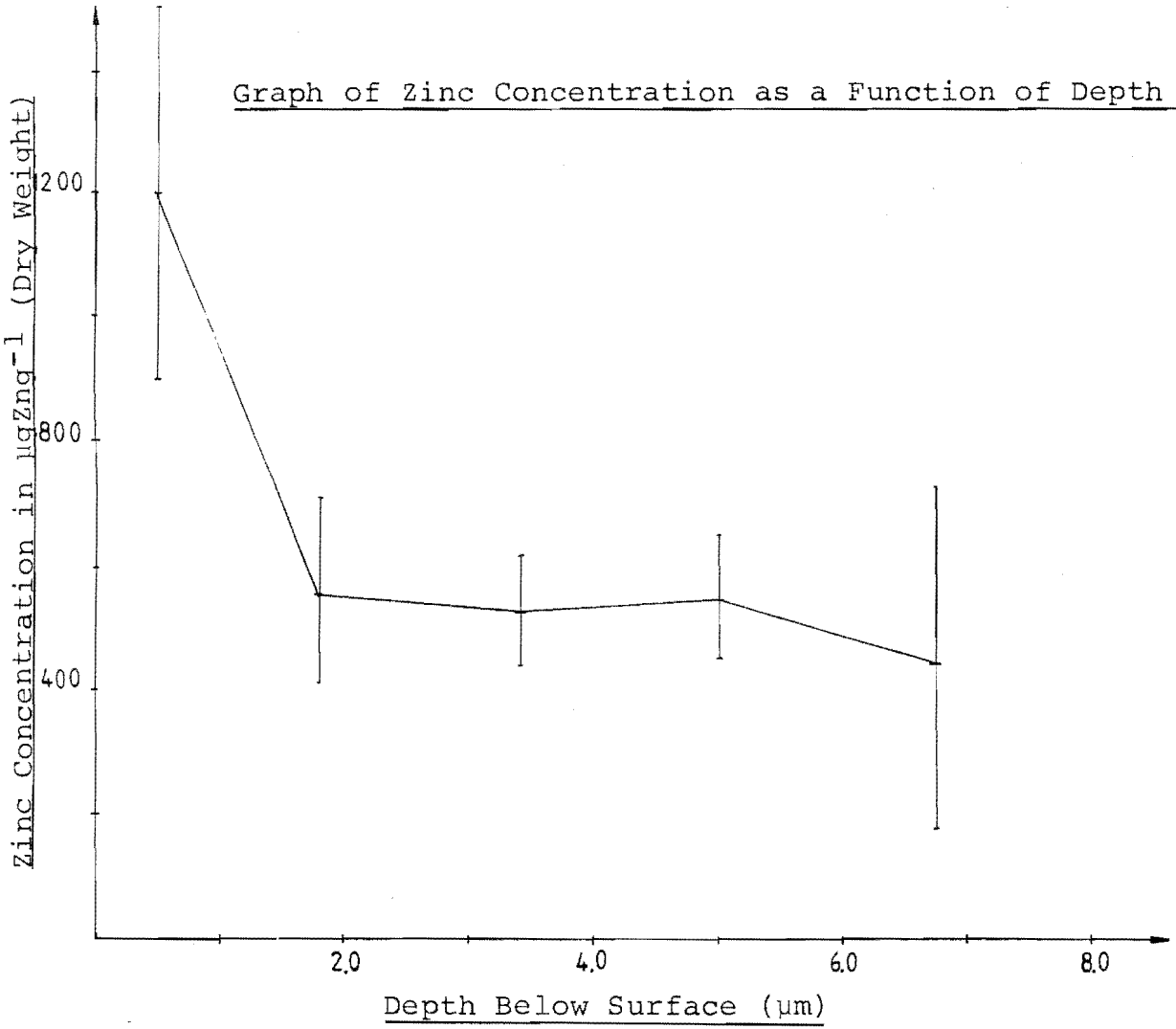


Table 6.7

Lead Concentration in Enamel with Depth (in $\mu gPbg^{-1}$ dry weight).

Tooth Sample Number	Lead Concentration	Depth (μm)	Tooth Sample Number	Lead Concentration	Depth (μm)
1	2400	0.31	2	1200	0.51
	790	1.07		490	1.74
	550	3.68		370	5.32
	380	4.85			
3	1950	0.14	4	1300	0.70
	270	2.11		850	3.70
	140	6.42		260	7.12
	82	12.93			
	50	33.95			
	16	39.93			

Table 6.7 cont.

Tooth Sample Number	Lead Concentration	Depth (μm)	Tooth Sample Number	Lead Concentration	Depth (μm)
5	500	0.65			
	330	1.95			
	160	3.52			

Note: (1) The calculation of depth of etch is explained in Section 6.3.2.

may vary across the sampled area. Also it may be possible that the etching acid works its way down between the crystals removing relatively more of the sorped ions compared with calcium. Hence the levels of the metals found could be high due to an effective concentrating effect during the etching.

6.3.3 The Effect of Tooth Type on Dentine Lead Concentrations.

In Chapter 5 when discussing the effect of tooth type on lead concentration, it was indicated that while there was considerable evidence for a pattern of lead concentration with tooth type for deciduous teeth, there was not such a clear picture for permanent teeth. Although differences between tooth types have been noted (17, 23), a discussion of permanent teeth is complicated by the presence of eight different types of teeth, and the possibility of differences for some tooth types between upper and lower jaws.

For deciduous teeth the lead concentration appears to depend on the time of eruption of the tooth and hence the tooth age. This produces the following order: lead concentrations in incisors>canines>molars, see Chapter 5 Section 5.3.5. For permanent teeth their time of eruption is such that all incisors or all molars can not be considered as one group, for example, first molars erupt at 6-7 years of age while second molars at 11-13 years and third molars 17-21 years. The approximate eruption time for each type of tooth is given in Table 6.8. The relative order of eruption is also the same as the order of formation of the teeth in the jaw.

Table 6.8

Formation and Eruption Age for Permanent Teeth.

Tooth Type	Onset of Formation (Years)	Eruption Age (Years)
Lower Jaw:		
Central Incisor	0.25-0.33	6-7
Lateral Incisor	0.25-0.33	7-8
Canine	0.33-0.50	9-10
First Premolar	1.75-2.00	10-12
Second Premolar	2.25-2.50	11-12
First Molar	at birth	6-7
Second Molar	2.50-3.00	11-13
Third Molar	8.00-10.00	17-21
Upper Jaw:		
Central Incisor	0.25-0.33	7-8
Lateral Incisor	0.83-1.00	8-9
Canine	0.33-0.50	11-12

Table 6.8 cont.

Tooth Type	Onset of Formation (Years)	Eruption Age (Years)
Upper Jaw:		
First Premolar	1.50-1.75	10-11
Second Premolar	2.00-2.25	10-12
First Molar	at birth	6-7
Second Molar	2.50-3.00	12-13
Third Molar	7.00-9.00	17-21

In an attempt to see if the trend in lead concentration in permanent teeth follows that of deciduous teeth, sixteen whole or partial sets of permanent teeth, from people living in Christchurch, New Zealand, were collected. Dentine from just below the neck of the tooth was used in the determination of lead concentration in the teeth. The results of these analyses are given in Table 6.9. The means for the near complete sets 1-8 were then found and the ratio of each individual tooth result to the mean for that set was obtained. A table of ratios is given in Table 6.10. The ratio for each type of tooth was then totalled over the eight sets of teeth and the mean ratio found, these being presented in Table 6.11. The values in Table 6.11 were then used to test the significance of the differences between tooth types.

The test for significance used was the Students t-test, and the results of this check are found in Table 6.12. The only occasion when the difference between upper and lower jaw was significant was that for central incisors, in all other cases differences were not significant. The data in Table 6.11 indicates that lead concentrations (from highest to lowest) are: first molars and lower central incisors; upper central incisors and lower lateral incisors; upper lateral incisors, canines, premolars and second molars; third molars; and that the differences are statistically significant (Table 6.12).

This follows, approximately, the pattern of eruption for permanent teeth (Table 6.7). Although the data is not given in Table 6.12 there was no significant difference between first and second premolars. This presence of a

Table 6.9

Lead Concentrations in Various Types of Teeth (in $\mu gPbg^{-1}$ dry weight).

Set No.	Lower Incisor	Upper Incisor	Lower Canine	Upper Canine	Lower Premolar	Upper Premolar	First Molar	Second Molar	Third Molar	Mean
1	20.5(C)	13.9(C)	17.4	13.1	14.8(F)	18.8(F)	22.6(L)	8.0(L)		
	23.5(C)		17.2	13.9	12.0(F)			8.9(L)		
	12.5(L)	10.6(L)			16.2(S)		10.8(U)	9.4(U)		13.0
	16.2(L)							8.1(U)		
2	59.2(C)		34.3		25.9(F)		40.1(L)	19.8(L)		
	33.0(L)		25.8		23.2(F)					
	30.6(L)									32.4
3	15.2(C)	16.4(C)	9.7	6.6	18.3(F)	12.5(F)	35.3(U)	14.8(L)		
	27.2(C)	14.9(C)	12.7			12.1(S)		16.9(L)		
	12.0(L)	8.3(L)						16.4(U)		14.8
	8.6(L)	8.0(L)								

Table 6.9 cont.

Set No.	Lower Incisor	Upper Incisor	Lower Canine	Upper Canine	Lower Premolar	Upper Premolar	First Molar	Second Molar	Third Molar	Mean
4	15.2(C)	12.9(C)	6.0	6.4	8.0(F)	6.0(F)	8.1(L)	5.7(L)	3.9(L)	
	11.5(C)	6.8(L)	6.7		8.8(F)	6.4(F)	7.0(L)	6.4(U)	2.8(L)	
	9.4(L)				8.2(S)	6.6(S)	16.2(U)	8.3(U)	4.9(U)	8.1
	7.0(L)				5.9(S)	5.3(S)	19.7(U)		5.6(U)	
5	31.2(C)	30.5(C)	21.7	22.1	18.7(F)	18.3(F)	26.4(L)	17.1(U)		
	30.3(C)	21.2(C)	20.4	15.0	18.8(F)	21.2(F)				
	29.2(L)	28.0(L)			21.5(S)	20.0(S)				23.3
	31.1(L)									
6	30.3(C)	30.5(C)	21.2	20.4	24.0(F)		24.0(L)	20.2(U)	6.0(L)	
	30.3(C)		17.0	26.6	23.6(F)		25.1(U)		6.3(L)	
	26.4(L)								10.4(U)	21.0
	26.4(L)								9.7(U)	
7	18.9(C)	16.3(C)	9.2	11.6	9.1(F)	10.5(F)	35.6(L)	13.4(L)	6.3(L)	
	18.4(C)	16.8(C)	9.2	8.4	8.8(F)	9.0(F)	23.9(L)	12.1(L)	4.0(L)	

Table 6.9 cont.

Set No.	Lower Incisor	Upper Incisor	Lower Canine	Upper Canine	Lower Premolar	Upper Premolar	First Molar	Second Molar	Third Molar	Mean
7	15.7(L)	15.4(L)			9.5(S)	14.1(S)	22.9(U)	10.0(U)	2.5(U)	13.2
	15.3(L)	11.5(L)			9.0(S)	12.1(S)	28.3(U)	11.3(U)	2.7(U)	
8	17.0(C)	8.5(C)	6.4	6.3	8.2(F)	8.1(F)	8.4(L)	2.6(L)	3.4(L)	
	16.7(C)	8.0(C)	6.7	7.0	7.6(F)	6.8(F)	9.5(L)		3.3(L)	
	12.9(L)	8.9(L)			6.9(S)	3.7(S)	14.1(U)			8.3
	11.0(L)	11.6(L)			5.5(S)	4.4(S)	11.2(U)			
9					13.7(F)	11.5(F)	17.6(L)	13.1(L)	3.8(L)	
					14.7(F)	15.0(F)	21.6(L)	10.4(L)	5.1(L)	
					10.2(S)	11.7(S)	17.8(U)	12.5(U)	4.4(U)	
					11.4(S)	10.6(S)	16.1(U)		3.2(U)	
10	36.8(C)	28.7(C)		15.4		25.4(F)				
		24.1(C)				23.4(F)				
		22.3(L)				22.3(S)				
		20.8(L)								

Table 6.9 cont.

Set No.	Lower Incisor	Upper Incisor	Lower Canine	Upper Canine	Lower Premolar	Upper Premolar	First Molar	Second Molar	Third Molar	Mean
11		29.3(C)		21.6		23.4(F)	31.8(U)	25.1(U)		
						17.9(F)				
12	7.7(C)		9.0		4.3(S)					
	11.3(C)		5.5							
	7.7(L)									
	8.6(L)									
13		3.6(C)		8.6		9.6(F)	11.3(U)	6.3(U)	3.6(U)	
		7.3(C)		5.0		9.2(F)	8.0(U)	4.4(U)		
		8.8(L)				6.0(S)				
		8.7(L)				7.4(S)				
14		6.1(C)		5.0						
		5.8(C)		4.5						
		5.7(L)								
		5.0(L)								

Table 6.9 cont.

Set No.	Lower Incisor	Upper Incisor	Lower Canine	Upper Canine	Lower Premolar	Upper Premolar	First Molar	Second Molar	Third Molar	Mean
15		42.7(C)				23.3(F)	39.0(U)			
		36.7(C)				21.0(F)				
		27.6(L)				16.1(S)				
		16.3(L)				18.1(S)				
16		32.5(C)				41.7(F)		23.8(U)		
						28.8(F)				
						18.5(S)				
						19.0(S)				

Notes: (1) The mean for a set of teeth is only calculated if there is a balanced representation of all tooth types.

(2) Abbreviations used in this table are as follows:

(C) Central (L) Lateral, if under incisor, or Lower if under molar

(F) First (S) Second (U) Upper

Table 6.10

Ratio of Tooth Lead Concentration to Mean of Set for Various Types of Teeth.

Set No.	Lower Incisor	Upper Incisor	Lower Canine	Upper Canine	Lower Premolar	Upper Premolar	First Molar	Second Molar	Third Molar
1	1.58(C)	1.07(C)	1.34	1.01	1.14(F)	1.45(F)	1.74(L)	0.62(L)	
	1.81(C)	0.82(L)	1.32	1.07	0.92(F)		0.83(U)	0.68(L)	
	0.96(L)				1.25(S)			0.72(U)	
	1.25(L)							0.62(U)	
2	1.83(C)		1.06		0.80		1.24(L)	0.61(L)	
	1.02(L)		0.80		0.72(F)				
	0.94(L)								
3	1.03(C)	1.11(C)	0.66	0.45	1.24(F)	0.84(F)	2.39(U)	1.00(L)	
	1.84(C)	1.01(C)	0.86			0.82(S)		1.14(L)	
	0.81(L)	0.56(L)						1.11(U)	
	0.58(L)	0.54(U)							

Table 6.10 cont.

Set No.	Lower Incisor	Upper Incisor	Lower Canine	Upper Canine	Lower Premolar	Upper Premolar	First Molar	Second Molar	Third Molar
4	1.89(C)	1.60(C)	0.74	0.79	0.99(F)	0.74(F)	1.00(L)	0.71(L)	0.48(L)
	1.43(C)	0.84(L)	0.83		1.09(F)	0.79(F)	0.87(L)	0.79(U)	0.35(L)
	1.17(L)				1.02(S)	0.82(S)	2.01(U)	1.03(U)	0.61(U)
	0.87(L)				0.73(S)	0.66(S)	2.44(U)		0.69(U)
5	1.34(C)	1.31(C)	0.93	0.95	0.80(F)	0.79(F)	1.13(L)	0.73(U)	
	1.31(C)	0.91(C)	0.88	0.64	0.81(F)	0.91(F)			
	1.25(L)	1.20(L)			0.92(S)	0.86(S)			
	1.33(L)								
6	1.44(C)	1.45(C)	1.10	0.97	1.14(F)		1.14(L)	0.96(U)	0.29(L)
	1.44(C)		0.81	1.27	1.12(F)		1.19(U)		0.30(L)
	1.26(L)								0.49(U)
	1.26(L)								0.46(U)
7	1.44(C)	1.24(C)	0.70	0.88	0.69(F)	0.80(F)	2.71(L)	1.02(L)	0.48(L)
	1.40(C)	1.28(C)	0.70	0.64	0.67(F)	0.68(F)	1.82(L)	0.92(L)	0.30(L)

Table 6.10 cont.

Set No.	Lower Incisor	Upper Incisor	Lower Canine	Upper Canine	Lower Premolar	Upper Premolar	First Molar	Second Molar	Third Molar
7	1.19(L)	1.17(L)			0.72(S)	1.07(S)	1.74(U)	0.76(U)	0.19(U)
	1.16(L)	0.87(L)			0.68(S)	0.92(S)	2.15(U)	0.86(U)	0.21(U)
8	2.04(C)	1.02(C)	0.76	0.76	0.99(F)	0.97(F)	1.01(L)	0.31(L)	0.41(L)
	2.01(C)	0.96(C)	0.81	0.84	0.91(F)	0.82(F)	1.14(L)		0.40(L)
	1.55(L)	1.07(L)			0.83(S)	0.44(S)	1.69(U)		
	1.32(L)	1.39(L)			0.66(S)	0.53(S)	.132(U)		

Notes: (1) Abbreviations used in this table are as follows:

(C) Central.

(L) Lateral if under incisors, or Lower if under molars.

(F) First (S) Second (U) Upper

Table 6.11

Mean and Standard Deviation for the Ratio of Lead Concentration in a Tooth to the Mean Lead Concentration from the Set.

Tooth Type	Mean	Standard Deviation	Number in Sample
Incisors:			
Central(L)	1.59	0.30	15
Central(U)	1.18	0.22	11
Lateral(L)	1.12	0.24	16
Lateral(U)	0.94	0.29	9
All Central	1.42	0.33	26
All Lateral	1.06	0.27	25
All Lower	1.35	0.36	31
All Upper	1.07	0.27	20
Total	1.24	0.35	51
Canines:			
Lower Jaw	0.89	0.20	16
Upper Jaw	0.85	0.22	12

Table 6.11 cont.

Tooth Type	Mean	Standard Deviation	Number in Sample
Canines:			
Total	0.87	0.21	28
Premolars:			
Lower Jaw	0.90	0.20	23
Upper Jaw	0.83	0.21	18
Total	0.87	0.20	41
First Molars:			
Lower Jaw	1.38	0.56	10
Upper Jaw	1.75	0.55	9
Total	1.61	0.69	19
Second Molars	0.81	0.21	18
Third Molars	0.40	0.14	14

Notes: (1) Abbreviations used in this table are as follows:

(L) Lower (U) Upper

Table 6.12

Level of Significance Between Various Types of Teeth Using Students t-Test.

Type of Tooth	Level of Significance	Type of Tooth	Level of Significance
Lower Central Incisor:		Upper Central Incisor:	
Upper Central Incisor	<.001		
Lower Lateral Incisor	<.00005	Lower Lateral Incisor	N.S.
Upper Lateral Incisor	<.00005	Upper Lateral Incisor	<.05
All Central Incisor	N.S.	All Central Incisor	<.05
All Lateral Incisor	<.00002	All Lateral Incisor	N.S.
All Lower Incisor	<.05	All Lower Incisor	N.S.
All Upper Incisor	<.00002	All Upper Incisor	N.S.
All Incisor	<.0005	All Incisor	N.S.
All Canine	<.00002	All Canine	<.0005
All Premolar	<.00002	All Premolar	<.0002
All First Molar	N.S.	All First Molar	<.02

Table 6.12 cont.

Type of Tooth	Level of Significance	Type of Tooth	Level of Significance
Lower Central Incisor:		Upper Central Incisor:	
All Second Molar	<.00002	All Second Molar	<.0002
All Third Molar	<.00002	All Third Molar	<.00001
Lower Lateral Incisor:		Upper Lateral Incisor:	
Upper Lateral Incisor	N.S.		
All Central Incisor	<.002	All Central Incisor	<.0005
All Lateral Incisor	N.S.	All Lateral Incisor	N.S.
All Lower Incisor	<.02	All Lower Incisor	<.001
All Upper Incisor	N.S.	All Upper Incisor	N.S.
All Incisor	N.S.	All Incisor	<.01
All Canine	<.002	All Canine	N.S.
All Premolar	<.0005	All Premolar	N.S.
All First Molar	<.01	All First Molar	<.002
All Second Molar	<.0005	All Second Molar	N.S.

Table 6.12 cont.

Type of Tooth	Level of Significance	Type of Tooth	Level of Significance
Lower Lateral Incisor:		Upper Lateral Incisor:	
All Third Molar	<.00001	All Third Molar	<.00005
All Central Incisor:		All Lateral Incisor:	
All Lateral Incisor	<.0001		
All Lower Incisor	N.S.	All Lower Incisor	<.002
All Upper Incisor	<.0005	All Upper Incisor	N.S.
All Incisor	<.05	All Incisor	<.02
All Canine	<.00001	All Canine	<.01
All Premolar	<.00001	All Premolar	<.005
All First Molar	N.S.	All First Molar	<.005
All Second Molar	<.00001	All Second Molar	<.002
All Third Molar	<.00001	All Third Molar	<.00001
All Lower Incisor:		All Upper Incisor:	
All Upper Incisor	<.005		

Table 6.12 cont.

Type of Tooth	Level of Significance	Type of Tooth	Level of Significance
All Lower Incisor:		All Upper Incisor:	
All Incisor	N.S.	All Incisor	<.05
All Canine	<.00001	All Canine	<.01
All Premolar	<.00001	All Premolar	<.005
All First Molar	N.S.	All First Molar	<.005
All Second Molar	<.00001	All Second Molar	<.005
All Third Molar	<.00001	All Third Molar	<.00001
All Incisor:		All Canine:	
All Canine	<.00001		
All Premolar	<.00001	All Premolar	N.S.
All First Molar	<.05	All First Molar	<.00005
All Second Molar	<.00001	All Second Molar	N.S.
All Third Molar	<.00001	All Third Molar	<.00001

Table 6.12 cont.

Type of Tooth	Level of Significance	Type of Tooth	Level of Significance
All Premolars:		All First Molar;	
All First Molar	<.00005		
All Second Molar	N.S.	All Second Molar	<.00005
All Third molar	<.00001	All Third Molar	<.00001
All Second Molar:			
All Third Molar	<.00001		

Notes: (1) Group at top of column is compared with those below it.

pattern of lead concentration in dentine of permanent teeth varying with the type of tooth, and the pattern of variance being the same as that for tooth eruption and formation, is strongly suggestive that, for dentine at least, the lead has accumulated with age. Also the results indicate a similar pattern as for deciduous teeth.

6.3.4 Lead Concentrations in Deciduous Teeth of Children in Rural Canterbury, New Zealand.

Deciduous teeth were obtained from children living in: Oxford, Sheffield, Springfield, Darfield, Greta Valley, Cheviot, Parnassus and Scargill. These are all small rural farming areas in North Canterbury. The teeth were collected with the help of the School Dental Nurse at the pupil's primary school, after the child's parents had filled out a form giving permission. Information was also obtained on the child's age, the position of the tooth in the mouth, address, age of home and type of material, previous addresses the child has had in its life, and the child's sex.

The analytical method for this study was the same as that used by Fergusson et al. (1), which allows for comparisons to be made with urban children living in Christchurch, New Zealand. The results for lead concentration in dentine from rural children as compared with the results of Fergusson et al. (1) for Christchurch are given in Table 6.13, and the distributions are plotted in Figure 6.6. Log transformed data (as the distributions are log-normal) was used to test (Student t-test) for the level of significant difference between the rural population and

Table 6.13

Comparison of Lead Concentrations in Deciduous Teeth of Rural Children with Those Living in Christchurch.

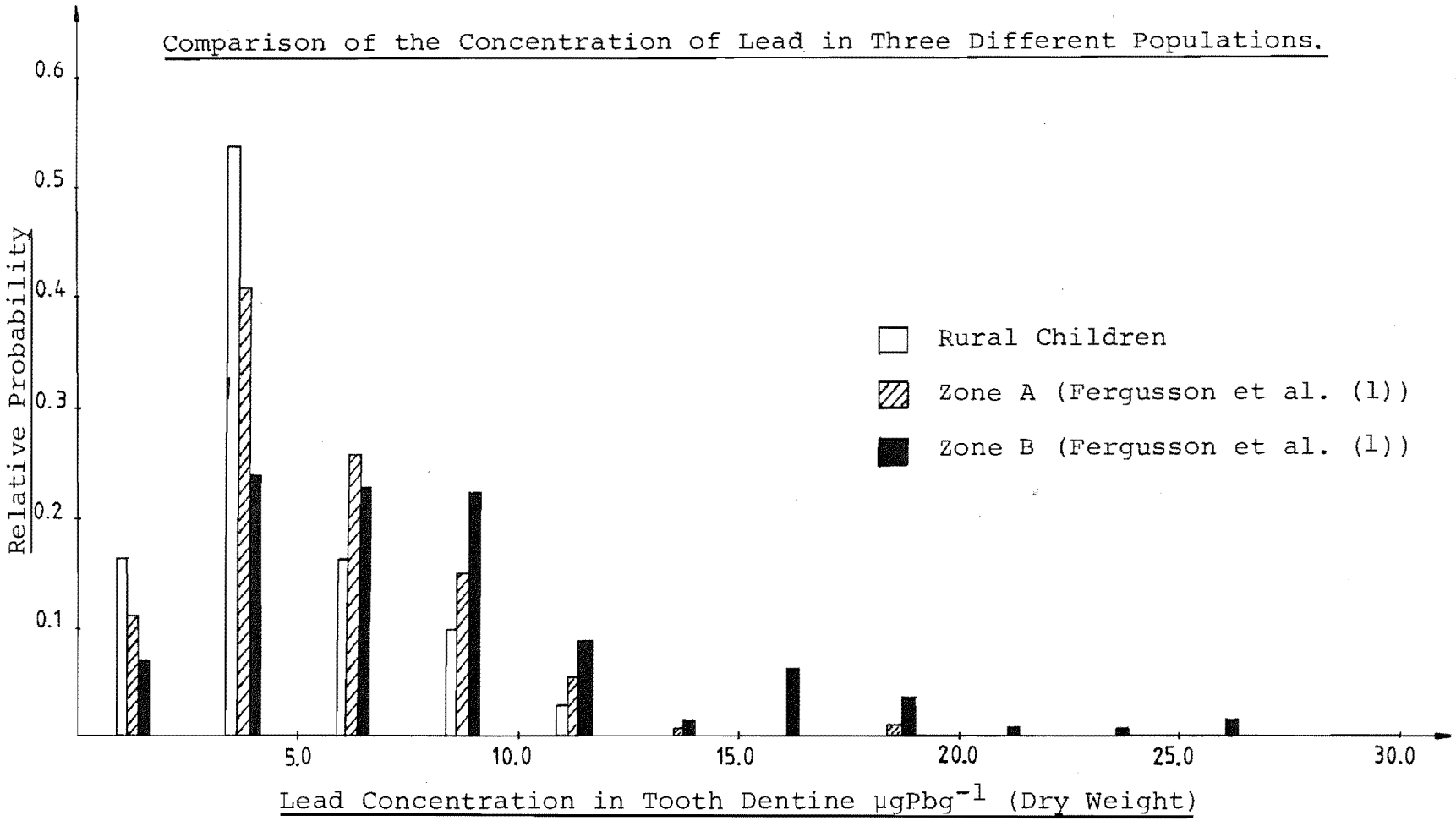
Population	Mean	S.D.	Median	$\log_{10}$Mean	$\log_{10}$S.D.	Range	No. in Population
Rural, North Canterbury	4.7	2.4	4.2	0.620	0.210	1.5-11.5	30
Christchurch, Zone A	5.6	3.2	4.8	0.683	0.232	1.4-25.0	145
Christchurhc, Zone B	6.9	4.6	5.7	0.827	0.280	1.4-27.0	144

Notes: (1) Data for Christchurch Zone A and Zone B, from Fergusson et al. (1)

(2) Lead Concentration in $\mu gPbg^{-1}$ dry weight.

(3) S.D. means standard deviation.

Figure 6.6


Comparison of the Concentration of Lead in Three Different Populations.
Relative Probability
0.6
0.5
0.4
0.3
0.2
0.1
Rural Children
Zone A (Fergusson et al. (1))
Zone B (Fergusson et al. (1))
5.0
10.0
15.0
20.0
25.0
30.0
Lead Concentration in Tooth Dentine µgPbg^{-1} (Dry Weight)

the children in Zone A in Christchurch (a suburban area of housing less than 30 years old). The level of significant difference between these populations was not very high $p<.15$. One reason for the low level of significance with this result is the small size of the rural population. When the rural population is compared with Zone B (an area of housing greater than 30 years old, or with an industrial presence), then the level of significant difference is $p<.0001$.

A more serious problem in making the comparison is that the type of teeth in the rural sample do not match the type of teeth in either of the two Christchurch samples (see Table 6.14). However, using the values in Table 6.14 for the Christchurch Zone A population and the values for average concentration of lead in various types of teeth for rural children from Table 6.15 only caused the mean to change from 4.7 to 4.9$\mu gPbg^{-1}$. This reduces the level of significance further, when comparing these populations ($p<0.20$), and this is not statistically significant.

When Fergusson et al. (1) compared Zones A and B, they argued that higher lead levels in Zone B were due to the presence of old housing, pre-world War II, and the presence of industry in the area. In the rural sample 47% of the children lived in wooden housing older than 30 years. However, there was no significant difference between those who lived in new, non-wooden housing, and those who lived in older wooden housing (median lead concentrations in dentine were 4.3 and 4.1$\mu gPbg^{-1}$ respectively). This could be due to the small size of the sample and is compounded by the problem that 30% of the sample had moved residence

Table 6.14

Comparison of Types of Teeth Present in Each Sample.

Population	Incisor	Canine	Molar	Total (N)
Rural	27%(8)	20%(6)	53%(16)	100%(30)
Christchurch, Zone A	52%(76)	22%(31)	26%(38)	100%(145)
Christchurch, Zone B	58%(84)	22%(31)	20%(29)	100%(144)

Notes: (1) Values are percentages of each type of tooth in the sample.

(2) Number in brackets is actual number of teeth in the sample.

(3) Data for Christchurch, Zone A and Zone B, from Fergusson et al. (1).

Table 6.15

Lead Concentrations in Various Types of Teeth from Rural Canterbury (in $\mu gPbg^{-1}$ dry weight).

Tooth Type	Number of Teeth	Mean	S.D.	Range
Incisor	8	4.8	1.6	2.1-6.6
Canine	6	6.4	3.0	2.3-10.0
Molar	16	4.0	2.3	1.5-11.5
Total	30	4.7	2.4	1.5-11.5

Note: (1) S.D. means standard deviation.

at least once since birth. This further compounds the difficulty in comparing the rural sample to the two Christchurch samples.

However, when looking at the distribution (Figure 6.6), two points should be considered. For the rural sample only 3% had dentine lead concentrations greater than $10\mu gPbg^{-1}$, while Zone A had 7.6% and Zone B had 24.3% of the sample above $10\mu gPbg^{-1}$. At the other extreme, for the rural sample 16% had lead concentration in dentine below $2.5\mu gPbg^{-1}$, while the values for Zones A and B are 11% and 7.5% respectively. Hence for the rural sample it appears that a greater percentage of the sample has low lead dentine concentrations when compared to Zones A and B.

This result of a greater proportion of children in rural environments having low concentrations of lead in teeth would suggest that their exposure to lead is less. The major difference between urban and rural children would appear to be traffic density and the associated emission of lead into the environment, and this would suggest that automotive lead emissions have some effect on the level of lead found in children's teeth.

6.4.1 Summary

From the work on permanent teeth presented in this chapter the following points have been made:

(1) That enamel may not be uniform in lead concentration. This is particularly true for incisors where lingual enamel concentration is higher than labial lead enamel

concentrations. Also it would appear that enamel obtained from near the neck of the tooth has a higher lead concentration than enamel obtained near the cutting tip of the tooth.

(2) That the ratio of lead concentration in enamel to lead concentration in dentine is not uniform for all teeth, and probably depends upon the age of the donor and the level of exposure to which the donor is subjected.

(3) That the concentration of lead in dentine is not constant, there being a significantly higher lead concentration in root dentine compared to coronal dentine.

(4) That the concentration of lead, copper, iron and zinc rapidly falls off from high concentration on the surface of dental enamel to low concentration in bulk enamel. It was not possible to establish the pattern for cadmium as the analytical technique employed was not sufficiently sensitive.

(5) That the lead concentration in dentine of various types of teeth varies in a manner reflecting the order of tooth eruption, with those teeth erupting first having higher lead concentration than those teeth which are the last to erupt.

The analysis of a group of deciduous teeth from a rural, North Canterbury population was found to have slightly lower lead concentrations in dentine than that from an industrialised and a suburban population from Christchurch. Although the results are not statistically significant

between the rural and suburban populations (Zone A) , this is predominantly due to the small size of the rural population.

6.4.2 Implications for the Use of Teeth as Indicators of Lead Burdens.

The results in the work show that the concentration of lead varies within the teeth, and within various zones of teeth and different types of teeth. This places stringent conditions on the use of teeth as indicators of lead concentrations in relation to the body burden. To minimise these effects the first step is to use only one type of tooth. The best tooth is probably the lower central incisor. In both permanent and deciduous teeth, this tooth has the highest lead concentration and this would reduce analytical problems. Also Delves et al. (26) found that deciduous teeth central incisors had the lowest variation between pairs.

An assumption, which seems justifiable from the present work, is that the distribution of lead is similar in deciduous and permanent teeth. If this is so, then the variation in lead concentrations within a tooth makes the choice of sample from a tooth very sensitive to the position sampled. Hence it may be better to lose information about lead distributions in teeth, when using teeth in a survey of lead burdens in a population, by using whole teeth. The only problem with this course of action is that for deciduous teeth the degree of reabsorption varies between donors and this could affect lead concentrations as it is mainly dentine that is reabsorbed. Since dentine appears to accumulate

lead with time, it could be used as an indicator to pick up situations where excessive lead exposure has occurred some time in the past of the donor.

However, in studies where only the extremes of a population are investigated then the variance in lead levels due to the type of tooth is less important. This variance in lead concentrations is less than 50% from mean values. Providing the zone of tooth is correctly removed, then variation within a zone is also of small magnitude. In these types of surveys then teeth may be used as an accumulative indicator of past lead burdens.

References.

1. Fergusson, J.E; Jansen, M.L; Sheat, A.W. Lead in deciduous teeth in relation to environmental lead. Environ. Technol. Lett., 1, 376-383, (1980).

2. Brudevold, F; Reda, A; Aasenden, R; Bakhos, Y. Determination of trace elements in surface enamel of human teeth by a new biopsy procedure. Arch. Oral Biol., 20, 667-673, (1975).

3. Brudevold, F; Aasenden, R; Srinivasian, B.N; Bakhos, Y. Lead in enamel and saliva, dental caries and the use of enamel biopsies for measuring past exposure to lead. J. Dent. Res., 56, 1165-1171, (1977).

4. Al-Naimi, T; Edmonds, M.I; Fremlin, J.H. The distribution of lead in human teeth, using charged particle activation analysis. Phys. Med. Biol., 25, 719-726, (1980).

5. Cohen, D.D; Clayton, E; Ainsworth, T. Preliminary investigations of trace element concentrations in human teeth. Nucl. Instrum. Methods Phys. Res., 188, 203-209, (1981).

6. Fremlin, J.H; Edmonds, M.I. The determination of lead in human teeth. Nucl. Instrum. Methods, 173, 211-215, (1980).

7. Shapiro, I.M; Needleman, H.L; Tuncay, O.C. The lead content of human deciduous and permanent teeth. Environ. Res., 5, 467-470, (1972).

8. Steadman, L.T; Brudevold, F; Smith, F.A; Gardner, D.E; Little, M.F. Trace elements in ancient Indian

teeth. J. Dent. Res., 38, 285-292, (1959).

9. Wipawin, W.T; Thomas, B.J; Khan, H.A. On the uses of solid state nuclear track detectors for low level α-counting. Int. J. Radiat. Phys. Chem., 6, 203-210, (1974).

10. Derise, N.L; Ritchey, S.J. Mineral composition of normal human enamel and dentin and the relation of composition to dental caries: II. Microminerals. J. Dent. Res., 53, 853-858, (1974).

11. Lappalainen, R; Knuuttila, M. The concentrations of Pb, Cu, Co, and Ni in extracted permanent teeth related to donors' age and elements in the soil. Acta Odontol. Scand., 39, 163-167, (1981).

12. Malik, S.R; Fremlin, J.H. A study of lead distribution in human teeth, using charged particle activation analysis. Caries Res., 8, 283-292, (1974).

13. Ahlberg, M; Akselsson, R. Proton-induced X-ray emission in the trace analysis of human tooth enamel and dentine. Int. J. Appl. Radiat. Isot., 27, 279-290, (1976).

14. Petersson, L.G; Lodding, A; Koch, G. Elemental microanalysis of enamel and dentin by secondary ion mass spectrometry (SIMS). Deciduous and permanent teeth from high and low flouride areas. Swed. Dent. J., 2, 41-54, (1978).

15. Shapiro, I.M; Mitchell, G; Davidson, I; Katz, S.H. The lead content of teeth. Evidence establishing new minimal levels of exposure in a living preindustrialized human population. Arch. Environ. Health, 30, 483-486, (1975).

16. Stack, M.V; Burkitt, A.J; Nickless, G. Characterization of teeth by trace elements. Int. J. Forens. Dent., 2, 62-65, (1974).

17. Pinchin, M.J; Newham, J; Thompson, R.P.J. Lead, copper and cadmium in teeth of normal and mentally retarded children. Clin. Chim. Acta, 85, 89-94, (1978).

18. Besic, F.C; Bayard, M; Wiemann, Jr. M.R; Burrell, K.H. Composition and structure of dental enamel: elemental composition and crystalline structure of dental enamel as they relate to its solubility. J. Am. Dent Assoc., 91, 594-601, (1975).

19. Brudevold, F; Steadman, L.T. The distribution of lead in human enamel. J. Dent. Res., 35, 430-437, (1956).

20. Cutress, T.W. A preliminary study of the microelement composition of the outer layer of dental enamel. Caries Res., 13, 73-79, (1979).

21. Lindh, U; Tveit, A.B. Proton microprobe determination of fluorine depth distributions and surface multielement characterization in dental enamel. J. Radioanal. Chem., 59, 167-191, (1980).

22. Little, M.F; Steadman, L.T. Chemical and physical properties of altered and sound enamel - IV. Trace element composition. Arch. Oral Biol., 11, 273-278, (1976).

23. Kaneko, Y. Trace metal in human teeth. II. Contents of lead, copper, cadmium and zinc in human teeth. Koku Eisei Gakkai Zasshi, 22, 79-97, (1972).

24. Needleman, H.L; Shapiro, I.M. Dentine lead levels in asymptomatic Philadelphia school children.

Subclinical exposure in high and low risk groups. Environ. Health Perspect., 7, 27-31, (1974).

24. Delves, H.T; Clayton, B.E; Carmichael, A; Bubear, M; Smith, M. An appraisal of the analytical significance of tooth-lead measurements as possible indices of environmental exposure of children to lead. Ann. Clin. Biochem., 19, 329-337, (1982).

25. Analytical methods in flame spectroscopy. Varian Techtron.

Chapter 7

Experimental Methods

7.1.1 Introduction

In previous chapters, experimental methods relevant to each section have been included with the chapter. However, atomic absorption spectroscopy used widely in this study will be discussed in this chapter, together with anodic stripping voltammetry and X-ray powder diffraction.

A list of the instrumentation used in this work is collected together at the end of this chapter in Table 7.2. The instrumental settings for these instruments are generally given in each chapter as the settings varied with the sample analysed.

7.2.1 The Handling of Solutions with Low Concentrations of Trace Metals

One of the major problems in trace element analysis is ensuring that the quantity of an element present in a sample is the same as the quantity analysed. One of the main ways an element can be lost is by absorption from solution onto the walls of containers. Considerable research has been undertaken in investigating the sorption of various ions onto container surfaces (1-3). The results suggest that lead (and many other elements) at concentrations in the $ngmL^{-1}$ range or lower, is strongly absorbed onto the container surfaces at neutral pH. Sorption processes are believed to be both physical electrostatic attraction and

chemisorption, and both of these processes are controlled by pH.

In order to minimise both ion-exchange and chemisorption processes, solutions were maintained in strongly acid conditions with nitric acid. The concentration of acid (approximately 0.5M but depending on the sample) was sufficient to block ion-exchange processes, probably by competition of hydrogen ions for the sorption sites on the container surface (2). The consensus of opinion is that if the pH<2, then absorption of lead ions onto borosilicate glass does not occur. Borosilicate glass was chosen as the container because in comparison with polyethylene, it does not require as high an acid concentration to prevent absorption losses. Also, the glass is more easily washed in acid, and absorbed ions are lost more quickly from its surface than from polyethylene. (Also, as dry ashing was used in some studies and handling was to be minimised, then glass was used as it could withstand the high temperatures).

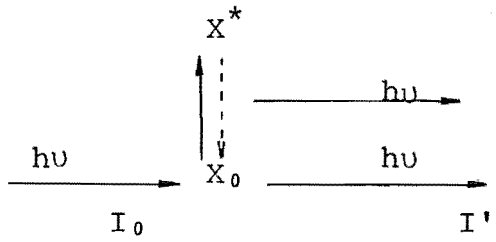
One problem encountered with surface absorption of trace elements was during the dry ashing of shellfish. Initially silica crucibles were used, but the recovery of added lead (200ng in nitric acid), after heating to 400^{o}C for 16 hours and digesting in heated 2M nitric acid, was found to be only 20-30%. However, when borosilicate glass beakers were used, recovery of lead was 97-102%.

The solvent used in the studies was 0.5M nitric acid. The only problem that occurred with this choice of acid is that nitrate can cause a shift in the potential wave for elements using ASV. However, metal nitrates under thermal decompositon yield oxides, and as these do not sublime or

vaporise easily, analysis using GFA-AAS can be relatively interference free, and reproducible.

7.3.1 Atomic Absorption Spectrophotometry

In atomic absorption spectrophotometry the presence of an atomic species is detected by the absorption of monochromatic light.



Radiation is absorbed by an atomic species producing an excited species which then radiates energy in all directions, dropping back to the ground state. Because the radiation is re-emitted in all directions it is not seen by the detector. For an absorption to take place the incident photons must have the same energy as an allowable transition for an atomic species. The strength of the absorption will then depend on both the transition probability and the absorption cross section for the particular atomic species and for the particular transition. This latter feature allows for different concentration ranges to be investigated.

As the absorption will only occur over a very narrow wavelength range the light has to be practically monochromatic. Two monochromatic sources are available for this purpose, the hollow-cathode lamp and the electrodeless discharge lamp. The hollow-cathode lamp works on the principle that after bombardment of the single element electrode with electrons, the atoms within the electrode

emit radiation characteristic of the electrode material. This restricts the technique to elements that can be used to make the electrodes.

The electrodeless discharge lamp depends upon electronic atomic spectra generation by the initial absorption of microwave radiation and atomic dissociation, followed by recombination and atomic photon emission as the atom returns to the ground state. To produce this type of lamp an element is required which has some vapour pressure at low temperatures ($\simeq 500^{o}C$). Electrodeless discharge lamps have a lower signal to noise ratio, and correspondingly a higher output intensity when compared with hollow-cathode lamps. However, hollow-cathode lamps for most elements are cheaper and more easily used in most commercial atomic absorption spectrophotometers, and were used throughout this study.

The lamps are not run continuously but are driven by a pulse voltage, this is so that a phase locked detector system can be used to limit the amount of noise present in the output. While this approach produces low noise, and has no disadvantages in measuring steady state concentrations, distortion can occur in non equilibrium systems, where concentrations change with time. This, to some extent can be minimised by having a high pulse rate in the lamp-detector system, and then the limiting factor becomes the response time of the detecting electronics. This problem of response time is most evident where deuterium background correction is employed.

Background correction is achieved with a continuous source, a deuterium lamp, and depends upon the simultaneous

measurement of light absorbed by the atomic species of the element being analysed and the measurement of other material in the path of the beam which absorbs over a broad range of frequencies. These broadband absorbances may be either molecular absorption, or merely scattering of the light by non absorbing molecular or atomic species. As the deuterium lamp is a broadband source, the absorbance that is measured by this lamp is predominantly that of broadband absorbances, while the absorbance of energy from the hollow-cathode lamp is that due to the analyte atoms as well as the broadband absorbances. Then, by subtracting the deuterium signal from the analyte element lamp signal, the analyte signal only may be obtained.

The use of background correction using a deuterium continuum lamp is satisfactory when the concentrations of analyte and background interference do not vary with time. In an ideal system there would be no problems with time variance of signals, as background and analyte signals would be measured simultaneously, but in practice the lamps are phased and a finite time delay occurs between signals, hence if the background changes rapidly during absorption the system will either over or under correct for background absorbance (4).

During the determination of lead concentrations in teeth in this study, a sample of "standard" dentine was employed, which consisted of a pool of powdered dentine. From standard addition analysis by GFA-AAS and ASV, and 80 determinations by GFA-AAS, the concentration of this sample was determined to be $10.3 \pm .0.2 \mu g Pbg^{-1}$. However, if automatic background correction was used the concentration

of lead was found to be $6.8 \pm 0.5 \mu g Pb g^{-1}$, showing a considerable over correction by the background correcting system.

7.3.2 Flame Atomised Atomic Absorption Spectrophotometry.

The simplest method of producing an atomic species is by placing the analyte sample into a hot flame, where thermal decomposition of any compounds in the analyte sample would occur, and this would generate a population of atoms of the analyte element. As the concentration of the analyte atoms within the flame depends on the flame temperature, choice of flame and flame conditions are important. The choice of flame and flame conditions also affect possible interferences in the flame.

One type of interference in flame atomisation occurs because the analyte atoms form refractory compounds within the flame. This may be controlled by either complexing the analyte element with an easily decomposed species, allowing easy generation of analyte atoms in the flame, or by using a hotter flame, in order to decompose most of the refractory compounds. While a complexing agent does not affect flame atomisation analysis, if the sample solution is also used for graphite furnace atomisation atomic absorption spectrophotometry (GFA-AAS) then the presence of a high concentration of complexing agent can cause scattering during the atomisation phase.

The use of a high temperature flame, while causing the thermal decomposition of all but the most refractory compounds, produces two side effects. Firstly, the increased

flame temperature causes higher noise levels in the output signal and hence an effective increase in the detection limit. The second effect of high flame temperature is thermal ionisation causing a lowering of the concentration of ground state atoms in the flame, and this also lowers the sensitivity of the technique.

7.3.3 Graphite Furnace Atomisation Atomic Absorption Spectrophotometry.

In flame atomisation AAS, the detection limit is determined by the flame noise and the amount of analyte present in the flame, which in turn is determined by the flame velocity and the maximum sustainable concentration of solution in the flame. With the use of graphite furnace atomisation the noise level is reduced considerably, and while the actual volume of solution used is much smaller than in flame atomisation, the instantaneous production of atoms within the graphite furnace is greater than for flame atomisation, allowing for the analysis of more dilute solutions. This increase in sensitivity varies by 10^2-10^3 times over that for flame atomisation, depending on the element.

In graphite furnace atomisation, the sample is placed into the furnace. In this study the volume of solution was 5μL, administered by a fixed volume Oxford micropipette. The control unit for the furnace has three stages. The first is the drying stage, in which the solvent, generally water, is volatilised, leaving the analyte and matrix deposited on the surface of the graphite furnace. The use of pyrolytic

graphite coating on the graphite cups and tubes minimises the absorption of the analyte solution onto the graphite of the furnace.

The second stage is the "ashing" process. The carbon furnace temperature is raised so that material can be decomposed and substances which are readily volatilised can be removed from the furnace. It is during this stage that most of the organic compounds decompose. While this is known as the "ashing" stage, ashing does not in fact occur with the graphite furnace atomiser used, as the graphite furnace is maintained in an atmosphere of oxygen free nitrogen. The nitrogen atmosphere is essential to minimise the decomposition of the graphite furnace at elevated temperature, but its pressure means that organic compounds undergo pyrolysis and not ashing.

The choice of ashing temperature is important because it is a compromise between removing as much of the matrix as possible and not losing any of the analyte at this stage in the analysis. While refractory elements like copper, iron and chromium present no problems during ashing as their volatilisation temperatures are high, on the other hand, lead, zinc and especially cadmium do present problems, as the loss of these elements may occur at the pyrolysis temperature of the organic matrix. In this case a lower "ashing" temperature must be used, together with a longer time to decompose any matrix.

The third stage is the atomisation process. In this stage the temperature of the furnace is increased so that the analyte material deposited on the graphite surface is volatilised off. The temperature of the furnace is adjusted

so that all the analysed material is removed out of the furnace, as well as the inorganic components of the matrix. There are two atomisation modes available on the graphite furnace atomiser used. Firstly, there is a step program where the voltage is suddenly raised and the furnace is held at the voltage for a fixed period of time. The second option is a ramp program where the voltage is ramped from the ashing voltage setting to a set voltage, at an adjustable voltage increase with time.

The step program where a rapid increase in voltage occurs, gives a higher peak concentration of analyte atoms in the incident light beam than the ramp mode, the increase in peak concentration being related to the rate of heating (5). However, in this mode more interference from the matrix can occur as the matrix is also volatilised at the same time. The step program is good if maximum sensitivity is required and the matrix includes a minimum of inorganic material.

The ramp program, because it is possible to control the rate of temperature rise, allows for a separation of analyte and matrix providing they volatilise at significantly different temperatures. The result appears as two or more distinct peaks in the output and the use of a deuterium continuum lamp allows for the assignment of each peak to either the analyte or the matrix. The use of the ramp program allows for the separation of the lead signal from that produced by the calcium phosphate matrix when analysing for lead in teeth.

A further consideration is whether to use signal (peak) height or area when calculating the absorbance of the

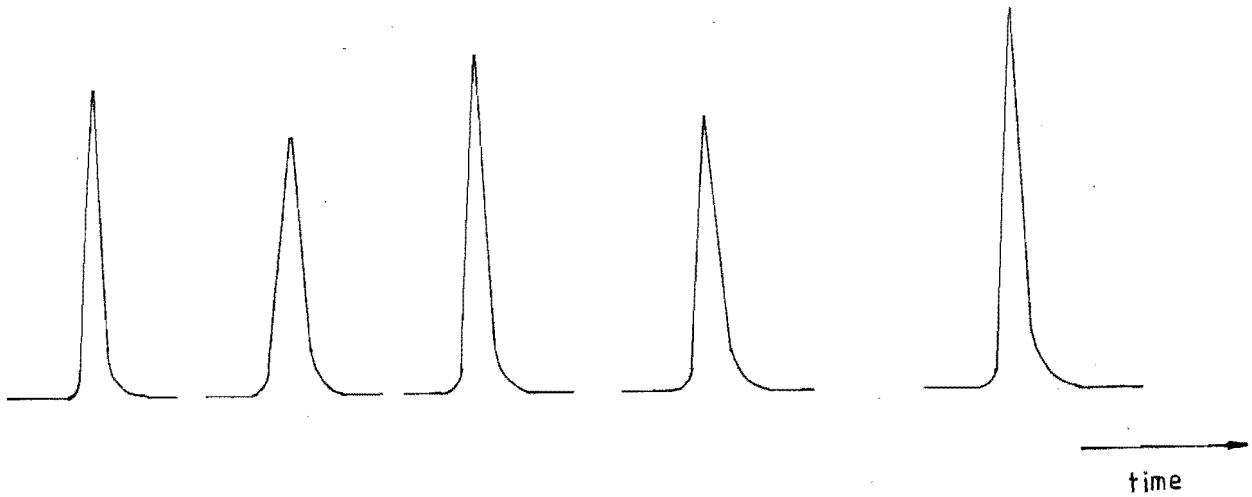
analyte signal. Peak height has the advantage that it is easy to measure. But the signal produced from the furnace is dependent on physical conditions within the furnace, the most important of these being the solvent spread in the furnace and the uniformity of the furnace heating. Since the uniformity of heating for the furnace is constant with runs then the time of release of analyte from the surface of the graphite furnace is dependent on solvent spread in the furnace, (this assumes that matrix effects are either not significant, or constant between samples).

It was found that for graphite tubes the peaks were consistent in profile so peak height was used as a measure of absorption, but when using graphite cups it was found necessary to use peak area. In Figure 7.1 is a reproduction of the output trace for the five successive determinations for lead in a standard containing 50ngPbmL^{-1}. While the peak height had a mean of 39 mm and a standard error of 11%, the peak area mean was 200 mm^2 with a standard error of 5%.

The final point to be considered with GFA-AAS is interference. The major reported interference is that of chloride ions, as lead chloride is more volatile than lead oxide and can be lost during the "ashing" stage (6). Two methods were used to combat this interference, which could occur in the analysis of lead in shellfish, as they came from a marine environment. The first method was to use nitric acid as the solvent so that lead was deposited on the graphite surface as lead nitrate and chloride ions were lost during the ashing stage as hydrochloric acid. The second method was to mix in 20% hydrogen gas into the inert

Figure 7.1

Output Trace for Five Determinations by Graphite Cup GFA-AAS for 50 ngPbmL^{-1}.



nitrogen gas supply. This is believed to help in removal of chloride during the "ashing" stage.

The proposed mechanism for the generation of lead atoms in the graphite furnace is first carbon reduction of lead oxide followed by generation of lead atoms (7).

$$PbO_{(s)} \longrightarrow Pb_{(l)} \longrightarrow \tfrac{1}{2}Pb_{2(g)} \longrightarrow Pb_{(g)}$$

For cadmium the generation of atoms occurs in the thermal decomposition of CdO and subsequent volatilisation of atomic cadmium (7).

$$CdO_{(s)} \longrightarrow \tfrac{1}{2}O_{2(g)}$$

$$CdO_{(s)} \longrightarrow Cd_{(l)} \longrightarrow Cd_{(g)}$$

7.4.1 Anodic Stripping Voltammetry

In anodic stripping voltammetry a hanging mercury drop is first placed in the solution to be analysed. A potential more negative than the reduction potential of the elements being investigated is then applied. This step is to concentrate metal ions in solution into the mercury drop, as at the drop reduction occurs and then amalgamation with the mercury. During this stage the solution is stirred so that local depletion around the drop does not occur. After a set time, which depends upon the concentration of analyte in solution, (for this work the time was two

minutes), the stirring is stopped and a pulsed or AC modulated ramped potential is passed across the electrodes so that oxidation of the metal within the mercury drop occurs. By using a pulsed wave form it is possible to sample the current at just before and at the end of the current pulse allowing a differential to be obtained. This allows for noise to be averaged and also for much greater amplification than DC anodic stripping voltammetry would allow.

The volume of solution used in these determinations was 20mL. The settings on the Princeton Applied Research Polarograph Analyser model 174A are given in Table 7.1.

In order to de-oxygenate the solution, a flow of oxygen free nitrogen was bubbled through the solution for 15 minutes prior to plating the mercury drop, and after the bubbling had ceased, a constant flow of oxygen free nitrogen was passed over the cell. In order to minimise contamination, a fresh mercury drop was extruded for each determination and all determinations were run in triplicate.

Two problems were found to occur with this method. Firstly, since not all of the organic material in the sample had been destroyed during the sample's dissolution in acid, it was found necessary to use the method of standard additions to calculate sample concentrations. This was accomplished with the addition of 6µL of a 100µgPbmL^{-1} solution to the sample solution. As this volume was very small compared with the sample volume (20mL), there was no need to correct for this change in volume. The second problem encountered arose from the high acid strength of

Table 7.1

Setting for Differential Pulsed Anodic Stripping Voltammetry using a Princeton Applied Research Polarograph Analyser Model 174A.

Mode	Differential Pulse
Initial Potential	1.20 Volts
Potential Scan	1.50 Volts
Scan Direction	Positive
Scan Rate	5.0 $mVsec^{-1}$
Modulation Amplitude	50 mV(PP)
Current Range	5 μA Full Scale
Output Offset	Off
Low Pass Filter	Off
Clock	Off
Display Direction	Negative

the sample solutions. The high acid concentrations (0.5M) were used to eliminate the possibility of analyte material being sorbed onto the walls of the container. However, at this high acidity the hydrogen over potential is lowered on the mercury electrode, making determination of zinc impossible in those solutions. A buffer was not used because of the problems of obtaining a trace element free buffer.

7.5.1 X-Ray Powder Diffraction

Since the requirements for good X-ray powder diffraction are a large number of small crystals, samples were finely ground between two frosted glass plates. Because of the small amount of some of the samples used for lead compound identification, a Debye-Scherrer camera was employed as samples as small as 2mg could be studied. One problem encountered using the powder camera was that as lead strongly absorbs X-rays, exposure times of approximately 24 hours were necessary for a satisfactory result.

Since the sample size for the clay analysis was much larger it was possible to use an X-ray powder diffractometer equipped with a scintillation counter, and this allowed samples to be run in approximately 1 hour.

Table 7.2

Equipment Used During This Study.

Method	Equipment
Atomic Absorption Spectrophotometry	Atomic Absorption Spectrophotometers: Varian AA-5 Varian AA-1475 (Both with deuterium background correctors). Graphite Furnace Atomiser: Varian CRA-63 (With graphite tubes and cups). Chart Recorders: Yokogawa Model No. 3046 Houston Instruments EB 5116-5
Differential Pulsed Atomic Stripping Voltammetry	Analyser: Princeton Applied Research Polarograph Analyser Model 174A Mercury Electrode: Princeton Applied Research Model Model 303 SMDE Chart Recorder: Princeton Applied Research Model RE 0074 X-Y Recorder

Table 7.2 cont.

Method	Equipment
Differential Thermal Analysis	Thermal Analyser: Aminco Model No, 4-4442A
	Chart Recorder: Aminco X-Y Recorder
X-Ray Powder Diffraction for Clays	X-Ray Generator: Philips PW 1050/25
	Copper X-Ray Lamp: Philips PW 1012/10
	X-Ray Diffractometer: Philips PW 1050/25
	Scintillation Counter: Philips PW 1362
X-Ray Diffraction for Lead Compounds	X-Ray Generator: Philips PW 1010/80
	Copper X-Ray Lamp: Philips PW 2223/20
	Debye-Scherrer Camera: Philips PW 1024
	Camera Motor: Philips PW 1033
Infrared Spectroscopy	Infrared Spectrometer: Pye Unicam SP-300

References.

1. Issaq. H.J; Zielinski, W.L. Jr. Loss of lead from aqeous solutions during storage. Anal. Chem., 46, 1328-1329, (1974).

2. Salim, R; Cooksey, B.G. Adsorption of lead on container surfaces. J. Electroanal. Chem., 106, 251-262, (1980).

3. Beneš, P; Sanyal, A.S; Krištofiková, Z; Obdržálek, M. Radiotracer study of lead adsorption on glass from very dilute aqueous solutions. Radiochim. Acta, 28, 35-38, (1981).

4. Garnys, V.P; Matousek, J.P. Correction for spectral interference with determination of lead in blood by nonflame atomic absorption spectrometry. Clin. Chem., 21, 891-893, (1975).

5. Holcombe, J.A; Rayson, G.D; Akerlind, N. Jr. Time and spatial absorbance profiles within a graphite furnace atomizer. Spectrochim. Acta, 37B, 319-330, (1982).

6. Matousek, J.P. Interferences in electrothermal atomic absorption spectrometry, their elimination and control. Prog. Analyt. Atom Spectrosc., 4, 247-310, (1981).

7. Chakrabarti, C.L; Wan, C.C; Teskey, R:J; Chang, S.B; Hamed, H.A; Bertels, P.C. Mechanism of atomization at constant temperature in capacitive discharge graphite furnace atomic absorption spectrometry. Spectrochim. Acta, 36B, 427-438, (1981).

Acknowledgment

I would like to acknowledge Dr. J.E. Fergusson, my supervisor, who has been both helpful and encouraging during this period of study. I would also like to thank Dr. H.J.K. Powell who acted as my supervisor while Dr. J.E. Fergusson was absent on study leave. I also wish to thank Dr. A.S. Campbell at the Soil Science Department, Lincoln College, for his help with DTA analysis of samples for lead compounds and Mr D. Hogarth of the Geology Department, University of Canterbury, for his assistance with clay type determinations by X-ray diffraction.

I also acknowledge the assistance of the Christchurch Meteorological Office who supplied the rainfall data and Mr K.O. Sullivan who supplied the sets of teeth used in this study. I also would like to thank the staff and postgraduate students of the Chemistry Department, University of Canterbury, for the many ways they have been of assistance to me during this period of study. I am grateful to the University of Canterbury for the support of a Teaching Fellowship.

Finally I wish to thank my family and friends for without their support and encouragement this course of study would have been far more difficult.